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(54) Low binding affinity Interleukin-12 beta receptors

(57) The present invention is directed to IL-12 receptor proteins comprising a complex of the beta1 receptor protein with the beta2 receptor protein, which complex is capable of binding to human IL-12 with high affinity. When expressed in host cells the nucleic acid gives rise to substantially homogeneous IL-12 receptor proteins. Further, the invention relates to antibodies capable of binding to cells expressing the IL-12 receptor molecules.

EP 0 759 466 A2

Description

This invention relates generally to Interleukin-12 receptors, especially to human Interleukin-12 receptors.

Interleukin-12 (IL-12), formerly known as cytotoxic lymphocyte maturation factor or natural killer cell stimulatory factor, is a 75-kDa heterodimeric cytokine composed of disulfide-bonded 40-kDa (p40) and 35-kDa (p35) subunits that has multiple biological activities including stimulation of the proliferation of activated T and NK cells (Gately, M. K., et al., 1991, *J. Immunol.*, 147:874) (Kobayashi, M., et al., 1989, *J. Exp. Med.*, 170:827), enhancement of the lytic activity of NK/LAK cells (Kobayashi, M., et al., 1989, *J. Exp. Med.*, 170:827; Stern, A.S., et al., 1990, *Proc. Natl. Acad. Sci. USA*, 87:6808), enhancement of cytolytic T-cell responses (Gately, M.K., et al., 1992, *Cell. Immunology*, 143:127), induction of interferon gamma by resting and activated T- and NK-cells (Kobayashi, M. et al., 1989, *J. Exp. Med.*, 170:827; Chan, S. H., et al., 1991, *J. Exp. Med.*, 173:869), and promotion of T₁-type helper cell responses (Manetti, R., et al., 1993, *J. Exp. Med.*, 177:1199; Hsieh, C.-S., et al., 1993, *Science* 260:547).

The biological activity of IL-12 is mediated by the binding of the IL-12 molecules to cell surface, or plasma membrane, receptors on activated T-and NK cells; however, the contributions of the individual subunits, p35 and p40, to receptor binding and signal transduction remain unknown. Studies with labeled IL-12 have shown that this binding occurs in a specific and saturable manner. IL-12 delivers a signal to target cells through a receptor that was initially characterised on phytohaemagglutinin (PHA)-activated CD4+ and CD8+ T-cells and on IL-2 activated CD56+ NK-cells (Chizzonite, R., et al., 1992, *J. Immunol.*, 148:3117; Desai, B., et al., 1992, *J. Immunol.*, 148:3125).

A survey of over 20 human cell lines belonging to the T-, B-, NK- and myelomonocytic lineages only identified a single CD4+, IL-2 dependent human T-cell line (Kit 225/K6) that constitutively expresses the IL-12 receptor and responds to IL-12 (Desai, B., et al., 1992, *J. Immunol.*, 148:3125; Desai, B., et al., 1993, *J. Immunol.* 150:207A). Freshly prepared PHA-activated peripheral blood mononuclear cells (PBMC) and the Kit 225/K6 cell line thus represent two convenient cell sources to study the biochemistry of the functional IL-12 receptor; there may be others.

Equilibrium binding experiments with ¹²⁵I-labeled IL-12 identified three sites with binding affinities for human IL-12 of 5-20 pM, 50-200 pM, and 2-6 nM on IL-12 responsive T-cells (Chizzonite, R., et al., 1994, *Cytokine* 6(5):A82a).

A cDNA encoding a low affinity IL-12 receptor was previously cloned (Chua, A., et al., 1994, *J. Immunology* 153:128; European Patent Application No. 0,638,644). Based on a previously suggested nomenclature (Stahl and Yancopoulos, 1993, *Cell* 74:587), the initially isolated human IL-12 receptor chain is called the beta1 chain.

The present invention is directed to IL-12 receptor proteins comprising a complex of the beta1 receptor protein with the beta2 receptor protein, which complex is capable of binding to human IL-12 with high affinity. When expressed in host cells the nucleic acid gives rise to substantially homogeneous IL-12 receptor proteins. Further, the invention relates to antibodies capable of binding to cells expressing the IL-12 receptor molecules.

Brief description of the drawings:

Figure 1: DNA sequence of human IL-12 receptor beta2 cDNA. (start codon = nucleotide 641; stop codon = nucleotide 3226.)(SEQ ID NO:1).

Figure 2: Amino acid sequence of human IL-12 receptor beta2 protein. (single underlined amino acid residues at the N-terminal sequence = signal peptide; amino acid nos. 623-646 = transmembrane area, marked by double underline; 9 potential N-linked glycosylation sites in the extracellular portion are marked by bold italics and are also underlined; conserved box 1 and 2 motifs in the cytoplasmic domain are shaded [amino acid residues nos. 667-669, 699-704, 786-798])(SEQ ID NO:2).

Figure 3: DNA sequence of human IL-12 receptor beta1 cDNA (start codon = nucleotide 65; stop codon = nucleotide 2050)(SEQ ID NO:3).

Figure 4: Amino acid sequence of human IL-12 receptor beta1 protein. (underlined amino acid residues of N-terminal sequence = signal peptide sequence; amino acid residues nos. 541 to 571 = transmembrane area marked by -----; 6 potential N-linked glycosylation sites in the extracellular portion marked by -----; conserved box 1 and 2 motifs in the cytoplasmic domain are marked by ----- [amino acid residues nos. 577 to 584 and 618 to 629])(SEQ ID NO:4).

Figure 5A: Scatchard analysis of recombinant human IL-12 binding to transfected COS cells expressing human IL-12 beta1 receptor protein.

Figure 5B: Scatchard analysis of recombinant human IL-12 binding to transfected COS cells expressing human IL-12 beta2 receptor protein.

Figure 5C: Scatchard analysis of recombinant human IL-12 binding to transfected COS cells coexpressing human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein.

Figure 6: Analysis of proliferation, in the presence of various concentrations of human IL-12, of Ba/F3 cells stably transfected with cDNA for human IL-12 beta1 receptor protein (-- ♦ --), with cDNA for human IL-12 beta2 receptor protein (-- ○ --), or with cDNA for both human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein (-- ● --), by measuring incorporation of tritiated thymidine.

The present invention relates to a low binding affinity interleukin-12 (IL-12) beta2 receptor protein, or a fragment thereof which has low binding affinity for IL-12, and when complexed with a IL-12 beta1 receptor protein forms a complex having high binding affinity to IL-12. In a preferred embodiment of the present invention the IL-12 beta2 receptor protein has an amino acid sequence which is substantially homologous to SEQ ID NO:2 or which is encoded by a nucleic acid which is substantially homologous to SEQ ID NO:1. In a more preferred embodiment the nucleic acid encoding the IL-12 beta2 receptor protein is encoded by a nucleic acid sequence that hybridises under stringent conditions to nucleic acid sequence SEQ ID NO:1 or which shares at least 80%, more preferably at least about 90%, and most preferably at least about 95% sequence homology with the polypeptide having the SEQ ID NO:1. Especially, the invention relates to the human IL-12 beta2 receptor protein having for example the amino acid sequence of SEQ ID NO:2 or allelic forms or variants thereof.

In addition, the invention relates to a complex capable of binding to IL-12 with high affinity, comprising the IL-12 beta2 receptor protein, or a fragment thereof as defined above complexed with human IL-12 beta1 receptor protein, or a fragment thereof which has low binding affinity for IL-12, and when complexed with a IL-12 beta2 receptor protein forms a complex having high binding affinity to IL-12.

In a preferred embodiment the above complex comprises an IL-12 beta1 receptor protein has an amino acid sequence which is substantially homologous to SEQ ID NO:4 or which is encoded by a nucleic acid which is substantially homologous to SEQ ID NO:3. In a more preferred embodiment the nucleic acid encoding the IL-12 beta1 receptor protein is encoded by a nucleic acid sequence that hybridises under stringent conditions to nucleic acid sequence SEQ ID NO:3 or which shares at least 80%, more preferably at least about 90%, and most preferably at least about 95% sequence homology with the polypeptide having the SEQ ID NO:3. Especially, the invention relates to the human IL-12 beta1 receptor protein having for example the amino acid sequence of SEQ ID NO:4 or allelic forms or variants thereof.

The present invention also relates to the above proteins or complexes which are soluble.

An aspect of the present invention is a protein or complex encoded by a nucleic acid which comprises two subsequences, wherein one of said subsequences encodes a soluble protein as defined above, and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region. The invention also includes proteins encoded by a first and a second nucleic acid, wherein the first nucleic acid comprises two subsequences, wherein one of said subsequences encodes a soluble fragment of an IL-12 receptor beta2 protein mentioned above and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region, and the second nucleic acid comprises two subsequences wherein one of said subsequences encodes a soluble fragment of a IL-12 receptor beta1 protein and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region.

The term "human IL-12 beta2 receptor protein" refers to (1) the protein of SEQ ID NO:2, or (2) any protein or polypeptide having an amino acid sequence which is substantially homologous to the amino acid sequence SEQ ID NO:2 and which has the following properties:

1) The protein or polypeptide has low binding affinity for human IL-12, and

2) The protein or polypeptide, when complexed with human beta1 receptor protein forms a complex having high binding affinity for human IL-12.

The term "human IL-12 beta1 receptor protein" refers to (1) the protein of SEQ ID NO:4, or (2) any protein or polypeptide having an amino acid sequence which is substantially homologous to the amino acid sequence SEQ ID NO:4 and which has the following properties:

1) The protein or polypeptide binds to has low binding affinity for human IL-12, and

2) The protein or polypeptide, when complexed with human beta2 receptor protein forms a complex having high binding affinity for human IL-12.

As used herein, the terms "IL-12 beta2 receptor protein" and "IL-12 beta1 receptor protein" includes proteins mod-

ified deliberately, as for example, by site directed mutagenesis or accidentally through mutations. The terms also includes variants which may be prepared from the functional groups occurring as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically acceptable, i.e. they do not destroy the activity of the protein and do not confer toxic properties on compositions containing it. These variants may include, for example, polyethylene glycol side-chains which may mask antigenic sites and extend the residence of the proteins in body fluids. Other variants include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of free amino groups of the amino acid residues formed with acyl moieties (e.g. alkanoyl or carbocyclic aryl groups) or O-acyl derivatives of free hydroxyl groups (for example that of seryl- or threonyl residues) formed with acyl moieties.

"Substantially homologous", which can refer both to nucleic acid and amino acid sequences, means that a particular subject sequence, for example, a mutant sequence, varies from the reference sequence by one or more substitutions, deletions, or additions, the net effect of which do not result in an adverse functional dissimilarity between the reference and subject sequences. For purposes of the present invention, sequences having greater than 80 %, more preferable greater than 90% homology and still more preferably greater than 95% homology, equivalent biological properties, and equivalent expression characteristics are considered substantially homologous. For purposes of determining homology, truncation of the mature sequence should be disregarded. Sequences having lesser degrees of homology, comparable bioactivity, and equivalent expression characteristics are considered substantial equivalents. Generally, homologous DNA sequences can be identified by cross-hybridisation under high stringency hybridisation conditions.

"A fragment of the IL-12 beta2 receptor protein" means any protein or polypeptide having the amino acid sequence of a portion or fragment of a IL-12 beta2 receptor protein, and which (a) has low binding affinity for IL-12, and (2) when complexed with a IL-12 beta1 receptor protein, forms a complex having high binding affinity for IL-12.

"A fragment of the IL-12 beta1 receptor protein" means any protein or polypeptide having the amino acid sequence of a portion or fragment of IL-12 beta1 receptor protein, and which when complexed with a IL-12 beta2 receptor protein, forms a complex having high binding affinity for IL-12.

A "soluble fragment" refers to a fragment of a IL-12 receptor protein having an amino acid sequence corresponding to all or part of the extracellular region of the protein and which retains the IL-12 binding activity of the intact IL-12 receptor protein. For example, a soluble fragment of a IL-12 beta2 receptor protein is a fragment of a IL-12 beta2 receptor protein having an amino acid sequence corresponding to all or part of the extracellular region of a human IL-12 beta2 receptor protein.

In accordance with the invention, a "complex" comprising IL-12 beta2 receptor protein, or a fragment thereof, complexed with IL-12 beta1 receptor protein, or a fragment thereof, may be expressed on the cell surface of the host cell. When expressed on the cell surface of the host cell, the complex has a high binding affinity for IL-12, whereas the IL-12 beta1 receptor protein and the IL-12 beta2 receptor protein alone each have a low binding affinity for IL-12.

In accordance with this invention, the IL-12 beta2 receptor protein may be expressed on the surface of a host cell.

In accordance with this invention, not only the IL-12 beta2 receptor protein may be obtained, but also fragments of IL-12 beta2 receptor protein which (1) have low binding affinity for IL-12 and (2) which when complexed with a IL-12 beta1 receptor protein forms a complex having high binding affinity. The fragments of IL-12 beta2 receptor protein may be obtained by conventional means, such as (i) proteolytic degradation of the human IL-12 beta2 receptor protein, (ii) chemical synthesis by methods routine in the art, or (iii) standard recombinant methods.

For purposes of the present invention, a human IL-12 receptor protein which has a high binding affinity for human IL-12 is a protein which binds to human IL-12 with a binding affinity of from about 5 to about 100 pM. For purposes of the present invention, a human IL-12 receptor protein which has a low binding affinity for human IL-12 is a protein which binds to human IL-12 with a binding affinity of from about 1 to about 10 nM. The binding affinity of a protein for IL-12 can be determined by conventional means, such as described in R. Chizzonite et al., 1992, J. Immunol., 148:3117 and as set forth in Example 5.

Fragments of IL-12 beta2 receptor protein can also be measured for binding affinity for IL-12 by conventional means, such as described in R. Chizzonite et al., 1992, J. Immunol., 148:3117 and as set forth in Example 5. The fragments of IL-12 beta2 receptor protein may be measured for binding affinity for IL-12 either alone or complexed with IL-12 beta1 receptor protein, or a fragment of IL-12 beta1 receptor protein which when complexed with a IL-12 beta2 receptor protein forms a complex having high binding affinity.

The present invention also relates to nucleic acids, e.g. DNA, cDNA, RNA, mRNA, etc. encoding the above proteins, for example a complex capable of binding to human IL-12 with high affinity, the complex comprising human IL-12 beta2 receptor protein, or a fragment thereof, and human IL-12 beta1 receptor protein, or a fragment thereof. Preferably these nucleic acids encode the human IL-12 beta2 receptor protein such as a nucleic acid having the SEQ ID NO:1 and/or the IL-12 beta1 receptor protein such as a nucleic acid having the SEQ ID NO: 3. The present invention also relates to recombinant vectors comprising an above nucleic acid, to expression vectors, and especially to expression vectors wherein the above nucleic acid is operably linked to control sequences recognised by a host cell. The invention includes eukaryotic and prokaryotic host cells transformed with one or more of the above vectors and especially to host

cells wherein the proteins or complexes are expressed on the surface of the host cells and to host cells wherein these cells proliferate in the presence of IL-12. The above host cells may be transformed with a first vector comprising a nucleic acid encoding the IL-12 receptor beta2 protein as defined above and a second vector comprising a nucleic acid encoding the IL-12 receptor beta1 protein as defined above or with a single vector comprising a nucleic acid encoding an IL-12 receptor beta2 protein and a nucleic acid encoding an IL-12 receptor beta1 protein.

As used herein, "nucleic acid" refers to a nucleic acid polymer, in the form of a separate fragment or as a component of a larger nucleic acid construct, which has been derived from a nucleic acid isolated at least once in substantially pure form, i.e., free of contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such sequences are preferably provided in the form a DNA or a cDNA with an open reading frame uninterrupted by internal nontranslated sequences, or introns, which are typically present in eukaryotic genes. However, it will be evident that genomic DNA containing the relevant sequences could also be used. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

"Expression vector" is a genetic element capable of replication under its own control, such as a plasmid, phage or cosmid, to which another nucleic acid segment may be attached so as to bring about the replication of the attached segment. It comprises a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters and enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences.

"Clone" is a group of identical DNA molecules derived from one original length of DNA sequence and produced by a bacterium or virus using genetic engineering techniques, often involving plasmids.

In addition, the invention refers to a purified, recombinant protein comprising two different polypeptide chains (a heterodimeric protein) which may be prepared by known methods. The two different polypeptide chains are each encoded by a different chimeric polynucleotide which has two nucleic acid subsequences fused in frame. The first nucleic acid subsequence of the first chimeric polynucleotide, located at its 5' end, is an isolated nucleic acid sequence encoding a soluble fragment of a IL-12 beta2 receptor protein. The second nucleic acid subsequence of the first chimeric polynucleotide, located at its 3' end, is an isolated nucleic acid sequence encoding all domains of a human Ig heavy chain (preferably IgG) except the first domain of the constant region. The first nucleic acid subsequence of the second chimeric polynucleotide, located at its 5' end, is an isolated nucleic acid sequence encoding a soluble fragment of IL-12 beta1 receptor protein. The second nucleic acid subsequence of the second chimeric polynucleotide, located at its 3' end, is an isolated nucleic acid sequence encoding all domains of a human Ig heavy chain (preferably IgG) except the first domain of the constant region.

The starting materials for the purified, recombinant proteins of the invention may be obtained by methods known in the art. In particular, on the basis of the DNA sequence coding for human IL-12 beta2 receptor protein described in Figure 1 and of the already known nucleic acid sequences for certain receptors, those partial nucleic acid sequences which code for a soluble fragment of IL-12 beta2 receptor protein can be determined and engineered from the DNA sequence coding for human IL-12 beta2 receptor protein described in Figure 1 using known methods, see Sambrook et al., „Molecular Cloning“, 2nd ed., Cold Spring Harbor Laboratory Press (1989). Similarly, on the basis of the DNA sequence coding for human IL-12 beta1 receptor protein described in Figure 3 and of the already known DNA sequences for certain receptors, those partial DNA sequences which code for a soluble fragment of human IL-12 beta1 receptor protein can be determined and engineered from the DNA sequence coding for human IL-12 beta1 receptor protein described in Figure 3 using known methods, see Sambrook et al., „Molecular Cloning“, 2nd ed., Cold Spring Harbor Laboratory Press (1989). Sources for isolated DNA sequences coding for constant domains of human immunoglobulins are known in the art and disclosed, for example, by Ellison et al., Nucl. Acid Res. 10, 4071-4079 (1982) for IgG₁ or Huck et al., Nucl. Acid Res. 14, 1779-1789 (1986) for IgG₃.

The isolated DNA sequence encoding the soluble fragment of human IL-12 beta2 receptor protein may be fused in frame, by known methods [Sambrook et al., „Molecular Cloning“, 2nd ed., Cold Spring Harbor Laboratory Press (1989)], to the isolated DNA sequence encoding all domains of a human Ig heavy chain (preferably IgG) except the first domain of the constant region. The resulting chimeric polynucleotide has located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta2 receptor protein and at its 3' end the isolated DNA sequence encoding all domains of the human Ig heavy chain except the first domain of the constant region.

Similarly, the isolated DNA sequence encoding the soluble fragment of human IL-12 beta1 receptor protein may be fused in frame, by known methods [Sambrook et al., „Molecular Cloning“, 2nd ed., Cold Spring Harbor Laboratory Press (1989)], to the isolated DNA sequence encoding all domains of a human Ig heavy chain (preferably IgG) except the first domain of the constant region. The resulting chimeric polynucleotide has located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta1 receptor protein and at its 3' end the isolated DNA sequence encoding all domains of a human Ig heavy chain except the first domain of the constant region.

The chimeric polynucleotides can then be integrated using known methods [Sambrook et al., „Molecular Cloning“,

2nd ed., Cold Spring Harbor Laboratory Press (1989)] into suitable expression vectors for expression in a non-human mammalian cell, such as a CHO cell. In order to make the homodimeric protein of the invention, the chimeric polynucleotide having located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta2 receptor protein is integrated into a suitable expression vector. In order to make the heterodimeric protein of the invention, the chimeric polynucleotide having located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta2 receptor protein and the chimeric polynucleotide having located at its 5' end the isolated DNA sequence encoding the soluble fragment of human IL-12 beta1 receptor protein are integrated into a single suitable expression vector, or two separate suitable expression vectors.

Preferably, the chimeric polynucleotide(s) is/are co-transfected together with a selectable marker, for example neomycin, hygromycin, dihydrofolate reductase (dhfr) or hypoxanthine guanine phosphoribosyl transferase (hprt) using methods which are known in the art. The DNA sequence stably incorporated in the chromosome can subsequently be amplified. A suitable selection marker for this is, for example, dhfr. Mammalian cells, for example, CHO cells, which contain no intact dhfr gene, are thereby incubated with increasing amounts of methotrexate after transfection has been performed. In this manner, cell lines which contain a higher number of the desired DNA sequence than the unamplified cells can be obtained.

The baculovirus expression system can also be used for the expression of recombinant proteins in insect cells. Posttranslational modifications performed by insect cells are very similar to those occurring in mammalian cells. For the production of a recombinant baculovirus which expresses the desired protein a transfer vector is used. A transfer vector is a plasmid which contains the chimeric polynucleotide(s) under the control of a strong promoter, for example, that of the polyhedron gene, surrounded on both sides by viral sequences. The transfer vector is then transfected into the insect cells together with the DNA sequence of the wild type baculovirus. The recombinant viruses which result in the cells by homologous recombination can then be identified and isolated according to known methods. When using the baculovirus expression system, DNA sequences encoding the immunoglobulin part have to be in the form of cDNA.

The expressed recombinant protein may be purified, for example, by known methods. For example, protein G affinity chromatography may be used to purify the homodimeric protein of the invention. Column chromatography, or any other method that enables differentiation between homodimeric proteins and heterodimeric proteins, may be used to purify the heterodimeric protein of the invention.

Expression of human IL-12 receptor protein having high binding affinity to human IL-12:

The cDNA of cells where the IL-12 receptor is known to be found is incorporated by conventional methods into a bacterial host to establish a cDNA library. PHA-activated PBMC and cells from the Kit 225/K6 cell line are examples of cell sources for the cDNA. RNA from the cells is extracted, characterised, and transcribed into single stranded cDNA by conventional methods. The single stranded cDNA is converted into double stranded cDNA by conventional methods. The double stranded cDNA is incorporated by conventional techniques into an expression vector, such as pEF-BOS. The plasmid DNA from the expression vector is then incorporated into a bacterial host by conventional methods to form a library of recombinants.

The cDNA library is screened by conventional expression screening methods, as described by Hara and Miyajima, 1992, EMBO, 11:1875, for cDNA's which when expressed with cDNA's for the human IL-12 beta1 receptor protein, give rise to a high affinity human IL-12 receptor. A small number of clones from the library are grown in pools. DNA is extracted by conventional methods from the pools of clones. The DNA extracted from a pool of clones is then transfected by conventional methods, along with a small amount of DNA from a plasmid containing the cDNA encoding the human IL-12 beta1 receptor protein, into non-human host cells. The non-human host cells are preferably mammalian, such as a COS cell. Labeled recombinant human IL-12 is then added to the non-human host cells previously transfected as described above and the binding signal of the pool is determined. This process is repeated for each pool. The pools showing a positive binding signal for IL-12 may then be subsequently broken down into smaller pools and assayed in the above manner until a single clone is selected which shows a positive binding signal.

The plasmid DNA from the selected clone is sequenced on both strands using conventional methods, such as an ABI automated DNA sequencer in conjunction with a thermostable DNA polymerase and dye-labeled dideoxynucleotides as terminators. Amino acid sequence alignments may be run as described by M. O. Dayhoff et al., Methods Enzymology 91:524 (1983) with the mutation data matrix, a break penalty of 6 and 100 random runs.

The DNA from the selected clone is then co-transfected by conventional methods with DNA from a plasmid containing the cDNA encoding the human IL-12 beta1 receptor protein into a non-human host cell, preferably a non-human mammalian cell such as a COS cell or a Ba/F3 cell.

Alternatively, by conventional recombinant methods, a plasmid may be engineered which contains transcription units (promoter, cDNA, and polyA regions) for both human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein. Plasmid DNA is transfected by conventional methods into a non-human host cell, preferably a non-human mammalian cell such as a COS cell or a Ba/F3 cell.

In accordance with this invention, DNA may be isolated which encodes human IL-12 beta2 receptor protein, or a

fragment thereof, which fragment (1) has low binding affinity for human IL-12 and (2) when complexed with human IL-12 beta1 receptor protein, forms a complex having high binding affinity for human IL-12.

An isolated nucleic acid sequence refers to a nucleic acid polymer, in the form of a separate fragment or as a component of a larger nucleic acid construct, which has been derived from nucleic acid isolated at least once in substantially pure form, that is, free of contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such sequences, e.g. DNA, are preferably provided in the form of an open reading frame uninterrupted by internal nontranslated sequences, or introns, which are typically present in eukaryotic genes. Genomic DNA containing the relevant sequences could also be used as a source of coding sequences. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

In accordance with this invention, a mammalian cell having the human IL-12 beta2 receptor protein or the complex expressed on its surface and which proliferates in response to human IL-12 is useful for determining IL-12 bioactivity. For example, such cells are useful for determining whether a given compound inhibits biological activity of human IL-12 or is an IL-12 agonist.

In addition, through the ability to express the human IL-12 beta2 receptor protein on a non-human mammalian cell surface, we can also express fragments of the human IL-12 beta2 receptor protein, and can determine whether these fragments, when complexed with the beta1 subunit, or an active fragment thereof, have the same properties and high binding affinity for IL-12 as the intact complex.

Isolated DNA encoding the human IL-12 beta2 receptor protein may be used to make a purified, recombinant protein which is soluble, and which binds to IL-12 with the same affinity as human IL-12 beta2 receptor protein. The isolated DNA encoding the human IL-12 beta2 receptor protein may also be used to make a purified, recombinant protein which is soluble, and which binds to IL-12 with the same affinity as the recombinant human IL-12 receptor complex of the beta1 receptor protein with the beta2 receptor protein [See, for example, Charnow, S. M. et al., Trends in Biotechnology, Vol. 14, 52-60(1996)].

Such purified, recombinant proteins, which bind to human IL-12, are useful for preventing or treating pathological conditions caused by excess or inappropriate activity of cells possessing IL-12 receptors, by inhibiting binding of IL-12 to such cells. Pathological conditions caused by excess activity of cells possessing IL-12 receptors include autoimmune dysfunctions, such as without limitation rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis.

A purified, recombinant protein which is soluble, and which binds to IL-12 with the same affinity as human IL-12 beta2 receptor protein is the fusion of a soluble fragment of human IL-12 beta2 receptor protein and a human Ig heavy chain (such as IgG, IgM or IgE, preferably IgG) having all domains except the first domain of the constant region. This recombinant protein, which is homodimeric, is encoded by a chimeric polynucleotide which has 2 DNA subsequences fused in frame. The first DNA subsequence, at the 5' end of the chimeric polynucleotide, is an isolated DNA sequence encoding a soluble fragment of human IL-12 beta2 receptor protein. The second DNA subsequence, located at the 3' end of the chimeric polynucleotide, is an isolated DNA sequence encoding all domains of a human heavy chain Ig (preferably IgG) except the first domain of the constant region. The desired recombinant protein can be generated by transfection of the chimeric polynucleotide into a non-human mammalian cell, such as a chinese hamster ovary (CHO) cell. The expressed recombinant protein can be purified, for example, by protein G affinity chromatography.

A purified, recombinant protein which is soluble, and which binds to IL-12 with the same affinity as the recombinant human IL-12 receptor complex of the beta1 receptor with the beta2 receptor is encoded by two chimeric polynucleotides which each have two DNA subsequences fused in frame. The first DNA subsequence of the first chimeric polynucleotide, located at the 5' end, is an isolated DNA sequence encoding a soluble fragment of human IL-12 beta2 receptor protein. The second DNA subsequence of the first chimeric polynucleotide, located at the 3' end, is an isolated DNA sequence encoding all domains of a human Ig heavy chain (for example, IgG, IgM, IgE, preferably IgG) except the first domain of the constant region. The first DNA subsequence of the second chimeric polynucleotide, located at the 5' end, is an isolated DNA sequence encoding a soluble fragment of human IL-12 beta1 receptor protein. The second DNA subsequence of the second chimeric polynucleotide, located at the 3' end, is an isolated DNA sequence encoding all domains of a human Ig heavy chain (for example, IgG, IgM, IgE, preferably IgG) except the first domain of the constant region. The desired recombinant protein may be generated by cotransfection of the two chimeric polynucleotides into a non human mammalian cell, such as a CHO cell. The expressed protein can be purified, for example, by any method that enables differentiation of homodimeric proteins from heterodimeric proteins, such as, for example, column chromatography.

In addition, the invention also relates to a process for the preparation of a protein mentioned above comprising the expression of an above mentioned nucleic acid in a suitable host cell.

In addition, monoclonal or polyclonal antibodies directed against the human IL-12 beta2 receptor protein, or fragments thereof, or the complex, may also be produced by known methods [See, for example, Current Protocols in Immunology, ed. by Coligan, J.E. et al., J. Wiley & Sons (1992)] and used to prevent or treat pathological conditions caused by excess activity of cells possessing IL-12 receptors by inhibiting binding of IL-12 to such cells.

Purified, recombinant proteins are useful for preventing or treating pathological conditions caused by excess or inappropriate activity of cells possessing IL-12 receptors by inhibiting binding of IL-12 to such cells.

"Purified", as used to define the purity of a recombinant protein encoded by the combined DNA sequences described above, or protein compositions thereof, means that the protein or protein composition is substantially free of other proteins of natural or endogenous origin and contains less than about 1% by mass of protein contaminants residual of production processes. Such compositions, however, can contain other proteins added as stabilizers, carriers, excipients or co-therapeutics. A protein is purified if it is detectable, for example, as a single protein band in a polyacrylamide gel by silver staining.

Purified recombinant proteins as described above (as well as antibodies to the human IL-12 beta2 receptor proteins and fragments thereof, and antibodies to the complex of this invention) can be administered in clinical treatment of autoimmune dysfunctions, such as without limitation rheumatoid arthritis, inflammatory bowel disease and multiple sclerosis.

The purified recombinant proteins described above (as well as antibodies to the human IL-12 beta2 receptor proteins and fragments thereof, and antibodies to the complex of this invention) can be used in combination with other cytokine antagonists such as antibodies to the IL-2 receptor, soluble TNF (tumor necrosis factor) receptor, the IL-1 antagonist, and the like to treat or prevent the above disorders or conditions.

In addition, the invention relates to pharmaceutical compositions comprising a protein or an antibody mentioned above and a pharmaceutically acceptable carrier. The pharmaceutical compositions may comprise a therapeutically effective amount of one or more cytokine antagonists.

Further, the invention relates to the use of a protein or an antibody mentioned above for the preparation of a medicament. These compounds are especially useful for the treatment of autoimmune dysfunction.

The dose ranges for the administration of the purified, recombinant proteins described above (as well as antibodies to the human IL-12 beta2 receptor proteins and fragments thereof, and antibodies to the complex of this invention) may be determined by those of ordinary skill in the art without undue experimentation. In general, appropriate dosages are those which are large enough to produce the desired effect, for example, blocking the binding of endogenous IL-12 to its natural receptor. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counter indications, if any, immune tolerance and other such variables, to be adjusted by the individual physician. The purified, recombinant proteins described above (as well as antibodies to the human IL-12 beta2 receptor proteins and fragments thereof, and antibodies to the complex of this invention) can be administered parenterally by injection or by gradual perfusion over time. They can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

The dose ranges for the administration of the IL-12 receptor proteins and fragments thereof may be determined by those of ordinary skill in the art without undue experimentation. In general, appropriate dosages are those which are large enough to produce the desired effect, for example, blocking the binding of endogenous IL-12 to its natural receptor. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counter indications, if any, immune tolerance and other such variables, to be adjusted by the individual physician. The expected dose range is about 1 ng/kg/day to about 10 mg/kg/day. The IL-12 receptor proteins and fragments thereof can be administered parenterally by injection or by gradual perfusion over time. They can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcohol/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replinishes, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, anti-microbials, anti-oxidants, chelating agents, inert gases and the like. See, generally, *Remington's Pharmaceutical Science*, 16th Ed., Mack Eds., 1980.

Assays for determining whether a given compound blocks IL-12 activity:

An aspect of the invention is the use of either the human IL-12 beta2 receptor protein or the complex of this invention as a screening agent for pharmaceuticals. In accordance with this invention, we can determine whether a given compound blocks human IL-12 activity or acts as an agonist of IL-12.

A biological activity of human IL-12 is the stimulation of the proliferation of activated T- and NK-cells. Proliferation of activated T-cells causes alloantigen-induced immune responses, such as allograft rejection (such as skin, kidney, and heart transplants) and graft-versus-host reaction in patients who have received bone marrow transplants. This biological activity of human IL-12 is mediated by the binding of the human IL-12 molecules to cell surface receptors on the

activated T-cells.

A compound that blocks human IL-12 activity would, therefore, inhibit the proliferation of activated T-cells and would be useful to treat or prevent alloantigen induced immune responses.

In order to determine if a compound blocks human IL-12 activity, first, a plurality of cells having expressed on their surface either the human IL-12 beta2 receptor protein or a fragment thereof, or the complex of the invention, which cells proliferate in the presence of human IL-12, is provided. The human IL-12 beta2 receptor protein or a fragment thereof binds to human IL-12 with low binding affinity, but when complexed with human beta1 receptor protein forms a complex having high binding affinity for human IL-12. The complex of the invention binds to human IL-12 with high binding affinity and comprises a complex of (1) human IL-12 beta2 receptor protein, or a fragment thereof which when complexed with

10 a human IL-12 beta1 receptor protein forms a complex having high binding affinity to human IL-12, and (2) human IL-12 beta1 receptor protein, or a fragment thereof which when complexed with a human IL-12 beta2 receptor protein forms a complex having high binding affinity to human IL-12. Second, the cells are contacted with human IL-12 and the given compound. Third, it is determined whether the presence of the given compound inhibits proliferation of the cells.

In order to determine if a compound is an agonist of human IL-12, first, a plurality of cells having expressed on their surface either the IL-12 beta2 receptor protein or a fragment thereof, or the complex of the invention, and which cells proliferate in the presence of human IL-12, is provided. The human IL-12 beta2 receptor protein or a fragment thereof binds to human IL-12 with low binding affinity, but when complexed with human beta1 receptor protein forms a complex having high binding affinity for human IL-12. The complex of the invention binds to human IL-12 with high binding affinity and comprises a complex of (1) human IL-12 beta2 receptor protein, or a fragment thereof which when complexed with

20 a human IL-12 beta1 receptor protein forms a complex having high binding affinity to human IL-12, and (2) human IL-12 beta1 receptor protein, or a fragment thereof which when complexed with a human IL-12 beta2 receptor protein forms a complex having high binding affinity to human IL-12. Second, the cells are contacted with human IL-12 or the given compound. Third, it is determined whether the presence of the given compound stimulates proliferation of the cells.

25 Examples of cells capable of expressing on their surface the complex, which cells proliferate in the presence of human IL-12 include, without limitation, PHA-activated PBMC, Kit 225/K6 cells, and Ba/F3 cells transfected with cDNA for both human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein. Examples of cells capable of expressing on their surface the human IL-12 beta2 receptor protein, or a fragment thereof, which cells proliferate in the presence of human IL-12 include, without limitation, Ba/F3 cells transfected with cDNA for human IL-12 beta2 receptor

30 protein.

In order to determine whether the presence of the given compound inhibits proliferation of the cells, the following procedure may be carried out. The human IL-12 responsive cells, having expressed on their surface the human IL-12 beta2 receptor protein, or a fragment thereof, or the human IL-12 receptor complex of the invention, are plated into wells of a microtiter plate. Human IL-12 is then added to some wells of the microtiter plate (standard wells) and allowed to react with the cells. The compound to be tested is added either before or simultaneously with human IL-12 to different wells of the microtiter plate (sample wells) and allowed to react with the cells. Any solvent used must be non-toxic to the cell. The proliferation of the cells is then measured by known methods, for example, labeling the cells after contact with human IL-12 and the compound (such as by incorporation of tritiated thymidine into the replicating DNA), measuring the accumulation of cellular metabolites (such as lactic acid), and the like. The proliferation of the cells of the standard wells is compared to proliferation of the cells of the sample wells. If the cells of the sample wells proliferate significantly less than the cells of the standard wells, the compound blocks IL-12 activity.

In order to determine whether the presence of the given compound simulates proliferation of the cells, the following procedure may be carried out. The human IL-12 responsive cells having expressed on their surface the human IL-12 beta2 receptor protein, or a fragment thereof, or the human IL-12 receptor complex of the invention are plated into wells of a microtiter plate. Human IL-12 is then added to some wells of the microtiter plate (standard wells) and allowed to react with the cells. The compound to be tested is added to different wells of the microtiter plate (sample wells) and allowed to react with the cells. Any solvent used must be non-toxic to the cell. The proliferation of the cells is then measured by known methods, for example, labeling the cells after contact with the compound (such as by incorporation of tritiated thymidine into the replicating DNA), measuring the accumulation of cellular metabolites (such as lactic acid), and the like. The proliferation of the cells of the standard wells is compared to proliferation of the cells of the sample wells. If the cells of the sample wells proliferate significantly more than cells that were not exposed to human IL-12, the compound is an agonist of human IL-12.

Accordingly, the present invention relates to a method for screening of compounds useful for inhibition of IL-12 activity or compounds useful as agonists of IL-12 activity, comprising contacting a compound suspected of inhibiting IL-12 activity or of being an agonist of IL-12 activity, to a protein mentioned above, followed by detection of the biological effect.

The following examples are offered by way of illustration, not by limitation.

EXAMPLES

MATERIALS AND METHODS:

5 1. Proteins, Plasmids and Strains

Recombinant human IL-12 (U. Gubler et al., 1991, Proc. Natl. Acad. Sci. USA., 88:4143) was obtained as described therein.

Recombinant human IL-2 (H.W. Lahm et al., 1985, J. Chromatog, 326:357) was obtained as described therein.

10 The plasmid pEF-BOS is based on a pUC 119 backbone and contains the elongation factor 1 alpha promoter to drive expression of genes inserted at the BstXI site (S. Mizushima and S. Nagata, Nucl. Acids Res., 1990, 18:5322).

The human IL-12 receptor beta1 cDNA in the plasmid pEF-BOS was obtained as described in A. Chua et al., 1994, J. Immunology 153:128 and in European Patent Application Publication No. 0638644.

15 Electrocompetent *E.coli* DH-10B (S. Grant et al., 1990, Proc. Natl. Acad. Sci USA 87:4645) was obtained from Bethesda Research Laboratory (Bethesda, Maryland).

2. Labeling of Human IL-12 with ^{125}I

20 Recombinant human IL-12 was labeled with ^{125}I as follows. Iodogen was dissolved in chloroform. 0.05 mg aliquots of iodogen were dried in 12 x 150 mm borosilicate glass tubes. For radiolabeling, 1.0 mCi Na $[^{125}\text{I}]$ was added to the iodogen-coated borosilicate glass tube, which also contained 0.05 ml of Tris-iodination buffer (25 mM Tris-HCl pH 7.5, 0.4 M NaCl and 1 mM EDTA) to form a ^{125}I solution. The ^{125}I solution was activated by incubating for 6 minutes at room temperature. The activated ^{125}I solution was transferred to a tube containing 0.05 to 0.1 ml recombinant human IL-12 (31.5 mg) in Tris-iodination buffer. The resulting mixture of the activated ^{125}I solution and the recombinant human IL-12 was incubated for 6 minutes at room temperature. At the end of the incubation, 0.05 ml of iodogen stop buffer (10 mg/ml tyrosine, 10% glycerol in Dulbecco's phosphate buffered saline (PBS), pH 7.40) was added and reacted for 3 minutes. The resulting mixture was then diluted with 1.0 ml Tris-iodination buffer containing 0.25% bovine serum albumin (BSA), and applied to a Bio-Gel P10DG desalting column for chromatography. The column was eluted with Tris-iodination buffer containing 0.25% BSA. 1 ml fractions containing the eluted peak amounts of labeled recombinant human IL-12 were combined. The combined fractions were diluted to 1×10^8 cpm/ml with 1% BSA in Tris-iodination buffer. Incorporation of ^{125}I into recombinant human IL-12 was monitored by precipitation with trichloroacetic acid (TCA). The TCA precipitable radioactivity (10% TCA final concentration) was typically in excess of 95% of the total radioactivity. The radiospecific activity of the labeled recombinant human IL-12 was typically 1000 to 2000 cpm/mole.

35 Example 1

Preparation of Human PHA-activated Lymphoblasts

40 Human peripheral blood mononuclear cells (PBMC) were isolated from blood collected from healthy donors as described in Gately et al., J. Natl. Cancer Inst. 69, 1245 (1982). The blood was collected into heparinized syringes, 45 diluted with an equal volume of Hank's balanced salt solution and layered over lymphocyte separation medium (LSM® obtained from Organon Teknica Corporation, Durham, North Carolina) in tubes. The tubes were spun at 2000 rpm for 20 minutes at room temperature. PBMC at the interface of the aqueous blood solution and the lymphocyte separation medium were collected. Collected PBMC were pelleted at 1500 rpm for 10 minutes through a 15 ml cushion of 20% sucrose in Hank's balanced salt solution. Pelleted PBMC were resuspended in tissue culture medium (1:1 mixture of RPMI 1640 and Dulbecco's modified Eagle's medium, supplemented with 0.1 mM nonessential amino acids, 60 mg/ml arginine HCl, 10 mM Hepes buffer, 2 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, 0.05 mM 2-mercaptoethanol, and 1 mg/ml dextrose) (TCM) plus 5% human serum and washed twice in TCM.

50 The PBMC were then activated to form lymphoblasts. In particular, $0.5 - 1 \times 10^6$ cells/ml in TCM plus 5% human serum plus 0.1% (v/v) PHA-P (Difco, Detroit, MI) were cultured for 3 days at 37°C in a 5% CO₂ atmosphere.

After three days, cell cultures were split 1:1 by volume in TCM plus 5% human serum and 50 U/ml recombinant human IL-2 to yield >95% T-cells. These cells were utilized for preparation of a cDNA library.

Example 2

55 Extraction and Characterization of RNA

PBMC isolated as in Example 1, activated with PHA for 2-3 days, were harvested and total RNA was extracted using Guanidine Isothiocyanate/Phenol as described by P. Chomczynski and N. Sacchi, Anal. Biochem., 162:156,

1987. PolyA⁺ RNA was isolated from the total RNA by one batch adsorption to oligo dT latex beads as described (K. Kurabayashi et al., Nucl. Acids Res. Symposium Series 19:61, 1988). The mass yield of this purification was about 4% of polyA⁺ RNA.

5 Example 3

cDNA Library

From the above polyA⁺ RNA, a cDNA library was established in the mammalian expression vector pEF-BOS as follows.

10 3 mg of polyA⁺ RNA were reverse transcribed into single stranded cDNAs using RNaseH minus reverse transcriptase in the presence of α-³²P-dCTP. The resulting single stranded cDNAs were converted into blunt ended double stranded cDNAs as described by U. Gubler and A. Chua, Essential Molecular Biology Volume II, T.A. Brown, editor, pp. 39-56, IRL Press 1991. BstXI linkers (A. Aruffo and B. Seed, Proc. Natl. Acad. Sci (USA) 84, 8573, 1987) were ligated 15 to the resulting double stranded cDNAs.

15 CDNA molecules having a size of greater than 800 base pairs (bp) were selected by size exclusion chromatography as follows. A Sephadex SF 500 column (0.8 x 29 cm) was packed by gravity in 10 mM Tris-HCl pH 7.8 - 1 mM EDTA - 100 mM NaAcetate. The radioactive cDNA with added BstXI linkers was applied to the column and 0.5 ml fractions were collected. The size distribution of radioactive cDNA was determined by performing electrophoresis on a small aliquot of 20 each fraction on a 1% agarose gel, drying the gel, and visualizing the size by exposure of the gel to X-ray film. cDNA molecules larger than 800 bp were size selected in this fashion.

25 The selected cDNA molecules were pooled and concentrated by ethanol precipitation. The pooled and concentrated selected cDNA molecules were subsequently ligated to the plasmid pEF-BOS as follows. The plasmid had been restricted with BstXI and purified over two consecutive 1% agarose gels. 300 ng of the restricted and purified plasmid DNA were ligated to 30 ng of size selected cDNA in 60 ml of ligation buffer (50 mM Tris-HCl pH 7.8 - 10 mM MgCl₂ - 10 mM DTT - 1 mM rATP - 25 mg/ml BSA) at 15°C overnight.

The following day, the plasmid ligated with the size selected cDNA was extracted with phenol. 6 mg of mussel glycogen were added to the resulting extract, and the nucleic acids were precipitated by ethanol. The resulting precipitate was dissolved in water and the nucleic acids again were precipitated by ethanol, followed by a wash with 80% ethanol. 30 A pellet was formed from the precipitated and washed nucleic acids. The pellet was dissolved in 6 ml of water. 1 ml aliquots of the dissolved pellet were subsequently electroporated into *E.Coli* strain DH-10B. Upon electroporation of 5 parallel aliquots, a library of about 10 million recombinants was generated.

35 Example 4

Expression Screening for cDNAs Encoding High Affinity IL-12 Receptors

The library was screened according to the general expression screening method described by Hara and Miyajima, 1992, EMBO, 11:1875.

40 Pools of about 100 *E.coli* clones from the above library were grown and the plasmid DNA was extracted from the pools by conventional methods. 2 x 10⁵ COS cells were plated per 35 mm culture well. COS cells were transfected with a transfection cocktail using the standard DEAE dextran technique described in "Molecular Cloning, a Laboratory Manual", 2nd Ed., J. Sambrook et al., Cold Spring Harbor Laboratory Press, 1989 ("Molecular Cloning"). The transfection cocktail contained (1) 1 mg of plasmid DNA extracted from the *E.Coli* clone pools derived from the above library, and 45 (2) 0.1 mg of pEF-BOS plasmid DNA containing the human IL-12 receptor beta1 cDNA.

3 days after transfection, the wells of COS cells were incubated with 10 pM labeled human recombinant IL-12 (specific activity = 1000-2000 cpm/mole) for 90 minutes at room temperature. The labeled human recombinant IL-12 was removed, and the COS cell monolayer was washed for one hour three times with binding buffer (RPMI 1640, 5% fetal bovine serum (FBS), 25 mM HEPES pH 7) to further select for COS cells expressing high affinity IL-12 receptors only (the binding of the IL-12 ligand to the low affinity sites was further reduced because the low affinity sites have a higher dissociation rate). Subsequently, the cell monolayers were lysed and counted in a gamma counter. After screening 440 pools (representing about 44,000 clones), one pool consistently showed a positive binding signal (300 cpm over 100 cpm background). From this pool, a single clone was subsequently isolated by sib-selection. This single clone (B5-10) contained a cDNA insert of about 3 kb that was completely sequenced.

55 The cDNA insert of clone B5-10 was incomplete with regard to the protein coding region because it did not contain an in-frame stop codon. The cDNA library of Example 3 was rescreened by conventional DNA hybridization techniques with the cDNA insert from clone B5-10, as described in Molecular Cloning and by Grunstein and Hogness, 1975, Proc. Nat. Acad. Sci. USA., 72:3961. Additional clones were thus isolated and then partially sequenced. The nucleotide sequence of one clone (No. 3) was found to (i) overlap with the 3' end of the nucleotide sequence of clone B5-10, (ii)

extend beyond the nucleotide sequence of clone B5-10, and (iii) contain an in-frame stop codon.

This composite DNA sequence is shown in Figure 1 (SEQ ID NO:1). The deduced amino acid sequence for the encoded receptor protein is shown in Figure 2. Based on the previously suggested nomenclature of Stahl and Yancopoulos, 1993, Cell 74:587, we call this newly isolated human IL-12 receptor chain the beta2 chain.

5

Example 5

Binding Assays

10 COS cells (4.5×10^7) were transfected by electroporation using a BioRad Gene Pulser (250 mF, 250 volts) with either (1) 25 mg of the B5-10 plasmid DNA expressing recombinant human IL-12 beta2 receptor protein, (2) 25 mg of the pEF-BOS plasmid DNA expressing recombinant human IL-12 beta1 receptor protein, or (3) a mixture of 12.5 mg of the B5-10 plasmid DNA expressing recombinant human IL-12 beta2 receptor protein and 12.5 mg of the pEF-BOS plasmid DNA expressing recombinant human IL-12 beta1 receptor protein. The electroporated cells were plated in a 600 cm² culture plate, harvested after 72 hours by scraping, washed and resuspended in binding buffer.

15 The cells were assayed to determine affinities of the expressed IL-12 receptors for human IL-12. In particular, equilibrium binding of labeled recombinant human IL-12 to the cells was performed and analyzed as described by R. Chizzonite, et al., 1992, J. Immunol., 148:3117. Electroporated cells (8×10^4) were incubated with increasing concentrations of ¹²⁵I-labeled recombinant human IL-12 at room temperature for 2 hours. Incubations were carried out in duplicate or triplicate.

20 Cell bound radioactivity was separated from free labeled ¹²⁵I-IL-12 by centrifugation of the mixture of electroporated cells and ¹²⁵I-labeled recombinant human IL-12 through 0.1 ml of an oil mixture (1:2 mixture of Thomas Silicone Fluid 6428-R15 (A.H. Thomas) and Silicone Oil AR 200 (Gallard-Schlessinger)) at 4°C for 90 seconds at 10,000 x g to form a cell pellet in a tube. The cell pellet was excised from the tip of the tube in which it was formed, and cell bound 25 radioactivity was determined in a gamma counter.

Receptor binding data were analyzed and the affinities were calculated according to Scatchard using the method described by McPherson, J., 1985, Pharmacol. Methods, 14:213.

Example 6

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Production of IL-12 Responsive Cell Line

Wild-type Ba/F3 cells, an IL-3-dependent mouse pro-B cell (Palacios, R. et al., 1985, Cell 41:727) and Ba/F3 cells expressing human IL-12 beta1 receptor protein (Chua, A., et al., 1994, J. Immunology 153:128) were cotransfected with 35 (1) 80 mg of pEF-BOS plasmid DNA expressing recombinant human IL-12 beta2 receptor protein and (2) 8 mg of a plasmid expressing a hygromycin resistance gene (Giordano, T.J., et al., 1990, Gene 88:285) by electroporation using a BioRad Gene Pulser (960 mF, 400 volts).

All cells were resuspended at a density of 2×10^5 viable cells/ml in a growth medium of RPMI 1640, 10% FBS, glutamine (2mM), penicillin G (100 U/ml), streptomycin (100 mg/ml), and 10% conditioned medium from the WEHI-3 40 cell line (ATCC No. TIB 68, American Type Culture Collection, Rockville, Maryland). The WEHI-3 cell line is a source of IL-3. The resuspended cells were then incubated at 37°C under 5% CO₂ for 120 hours.

Cells were selected by their ability to grow in (1) the above growth medium in the presence of 1 mg/ml hygromycin or (2) an IL-12 containing growth medium of RPMI 1640, 10% FBS, glutamine (2mM), penicillin G (100 U/ml), streptomycin (100 mg/ml), and various concentrations (10, 50 or 250 ng/ml) of human IL-12.

45 Ba/F3 cells expressing human IL-12 beta1 receptor protein transfected with pEF-BOS plasmid DNA expressing recombinant human IL-12 beta2 receptor protein grew in the IL-12 containing growth medium, demonstrating that coexpression of human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein conferred human IL-12 responsiveness to the Ba/F3 cells.

Additionally, Ba/F3 cells expressing human IL-12 beta2 receptor protein grow in the IL-12 containing growth 50 medium, demonstrating that expression of human IL-12 beta2 receptor protein conferred human IL-12 responsiveness to the Ba/F3 cells.

Example 7

Effect of Human IL-12 on Transfected Ba/F3 Cell Lines

Ba/F3 cells (1) expressing human IL-12 beta1 receptor protein, (2) expressing human IL-12 beta2 receptor protein, or (3) coexpressing human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin G, 100 mg/ml streptomycin, and 2 mM L-glutamine at

2 x 10⁴ cells/well in Costar 3596 flat-bottom microplates for 24 hours. Various dilutions of human IL-12, as shown in Figure 6, were then added to the microplates and the cells were incubated for 42 hours at 37°C in a humidified atmosphere of 5% CO₂ in air. 50 ml of ³H-thymidine, 10 mCi/ml in culture medium, was then added to each well. The cultures were further incubated for 6 hours at 37°C. Subsequently, the culture contents were harvested onto glass fiber filters by means of a cell harvester. ³H-thymidine incorporation was measured by use of a liquid scintillation counter. All samples were assayed in quadruplicate.

Example 8

10 Sequence Analysis of IL-12 Receptor cDNA Clones and Encoded IL-12 Receptor Protein

The IL-12 beta2 receptor protein, composed of 862 amino acids and a calculated molecular weight of 97231, had the following features: N-terminal signal peptide, extracellular domain, transmembrane domain and cytoplasmic tail. The classical hydrophobic N-terminal signal peptide is predicted to be 23 amino acids in length. Signal peptide cleavage occurs mostly after the amino acids Ala, Ser, Gly, Cys, Thr, Gln (von Heijne, G., 1986, *Nucl. Acids Research*, 14:4683). For the IL-12 receptor, the cleavage could thus take place after Ala23 in the sequence shown in Figure 2, leaving a mature protein of 839 amino acids based on cleavage at Ala23. The extracellular domain of the receptor is predicted to encompass the region from the C-terminus of the signal peptide to amino acid No. 622 in the sequence shown in Figure 2. Hydrophobicity analysis shows the area from amino acid No. 623 to 646 to be hydrophobic, as would be expected for a transmembrane anchor region. Charged transfer stop residues can be found at the N- as well as the C-terminus of this predicted transmembrane area. The extracellular domain of the receptor is thus 599 amino acids long and contains 9 predicted N-linked glycosylation sites. The cytoplasmic portion is 215 amino acids long (amino acid residue nos. 647 to 862).

Further analysis of the amino acid sequence shown in Figure 2 shows the human IL-12 beta2 receptor protein is a member of the cytokine receptor superfamily, by virtue of the sequence motifs [Cys132 -- Cys143TW] and [W305SKWS]. Comparing the sequence shown in Figure 2 to all the members of the superfamily by running the ALIGN program shows that the human IL-12 beta2 receptor protein has the highest homology to human gp130. The cytoplasmic region of the IL-12 receptor beta2 chain contains the box 1 and 2 motifs found in other cytokine receptor superfamily members, as well as three tyrosine residues. Phosphorylation of tyrosines is commonly associated with cytokine receptor signalling; the presence of these tyrosine residues underscores the importance of the IL-12 receptor beta2 chain in the formation of a functional IL-12 receptor. The IL-12 receptor beta1 chain does not contain any tyrosine residues in its cytoplasmic tail.

Example 9

35 Analysis of the Binding Assays

The results of the binding assays are shown in Figure 5.

As shown in Figures 5A and 5B, human IL-12 binds to recombinant IL-12 receptor beta1 or beta2 alone with an apparent affinity of about 2.5 nM. The binding data was described by a single site receptor model, corresponding to the low affinity component of the functional IL-12 receptor found on PHA-activated PBMC (R. Chizzonite et al., 1992, *J. Immunol.*, 148:3117; B. Desai et al., 1992, *J. Immunol.*, 148:3125).

In contrast to these results, as shown in Figure 5C, both high and low affinity IL-12 binding sites were generated upon cotransfection of COS cells with IL-12 receptor beta1 and beta2 plasmids. In this case, the binding data were described by a two receptor site model, with affinities of 50 pM and 5 nM.

Example 10

Effect of Human IL-12 on Transfected Ba/F3 Cell Lines

50 The results of the proliferation assay for the effect of human IL-12 on Ba/F3 cells (1) expressing human IL-12 beta1 receptor protein, (2) expressing human IL-12 beta2 receptor protein, and (3) coexpressing human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein are shown in Figure 6.

Cells that are transfected with cDNAs for both human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein respond to stimulation by human IL-12 by proliferating in a dose-dependent manner.

Additionally, cells that are transfected with cDNAs for human IL-12 beta2 receptor protein respond to stimulation by human IL-12 by proliferating in a dose-dependent manner.

Consequently, isolated cDNA (clone No. B5-10, SEQ.ID. No:1) coding for a type I transmembrane protein represents a second component of the IL-12 receptor (IL-12R beta2) found on normal human T-cells. The beta1 and beta2

chains each alone bind IL-12 only with low affinity ($K_d = 2-5 \text{ nM}$). Upon coexpression of beta1 and beta2, two affinity sites are observed, with K_d values of 50 pM and 5 nM.

Ba/F3 cells expressing human IL-12 beta2 receptor protein or coexpressing human IL-12 beta1 receptor protein and human IL-12 beta2 receptor protein are responsive to human IL-12.

5 The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

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SEQUENCE LISTING

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(1) GENERAL INFORMATION:

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(i) APPLICANT:

- (A) NAME: HOFFMANN-LA ROCHE AG
- (B) STREET: Grenzacherstrasse 124
- (C) CITY: Basle
- (D) STATE: BS
- (E) COUNTRY: Switzerland
- (F) POSTAL CODE (ZIP): CH-4002
- (G) TELEPHONE: 061-688 51 08
- (H) TELEFAX: 061-688 13 95
- (I) TELEX: 962292/965542 hlr ch

20

(ii) TITLE OF INVENTION: RECEPTORS FOR INTERLEUKIN-12

30

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

40

(2) INFORMATION FOR SEQ ID NO:1:

45

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4040 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double

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(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: cDNA

10 (iii) HYPOTHETICAL: NO

15 (iv) ANTI-SENSE: NO

20 (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 641..3226

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGCAGAGAAC	AGAGAAAGGA	CATCTGGAG	GAAAGTTCCC	TGATGGCTGT	CAACAAAGTG	60
CCACGTCTCT	ATGGCTGTGT	ACGCTGAGCA	CACGATTTTA	TCGGCCCTAT	CATATCTTGG	120
TGCATAAACG	CACCTCACCT	CGGTCAACCC	TTGCTCCGTC	TTATGAGACA	GGCTTTATTA	180
TCCGCATTTT	ATATGAGGGG	AATCTGACGG	TGGAGAGAGA	ATTATCTTGC	TCAAGGCGAC	240
ACAGCAGAGC	CCACAGGTGG	CAGAATCCCA	CCCGAGCCCG	CTTCGACCCG	CGGGGTGGAA	300
ACCACGGGCG	CCCGCCCCGGC	TGCGCTTCCA	GAGCTGAAC	GAGAAGCGAG	TCCTCTCCGC	360
CCTGCGGCCA	CCGCCCCAGCC	CCGACCCCG	CCCCGGCCCG	ATCCTCACTC	GCCGCCAGCT	420
CCCCCGCGCC	ACCCCGGAGT	TGGTGGCGCA	GAGGCGGGAG	GCGGAGGCCG	GAGGGCGGGC	480
GCTGGCACCG	GGAACGCCCG	AGCCCGGCA	GAGAGCGCGG	AGAGCGGCAC	ACGTGGCGCC	540

EP 0 759 466 A2

CAGAGCACCG GGGCCACCCG GTCCCCGCAG GCCCGGGACC GCGCCCGCTG GCAGGGCACA	600
5 CGTGGAAAGAA TACGGAGTTC TATACCAGAG TTGATTGTTG ATG GCA CAT ACT TTT	655
Met Ala His Thr Phe	
10 AGA GGA TGC TCA TTG GCA TTT ATG TTT ATA ATC ACG TGG CTG TTG ATT	703
Arg Gly Cys Ser Leu Ala Phe Met Phe Ile Ile Thr Trp Leu Leu Ile	
15 10 15 20	
20 AAA GCA AAA ATA GAT GCG TGC AAG AGA GGC GAT GTG ACT GTG AAG CCT	751
Lys Ala Lys Ile Asp Ala Cys Lys Arg Gly Asp Val Thr Val Lys Pro	
25 25 30 35	
25 TCC CAT GTA ATT TTA CTT GGA TCC ACT GTC AAT ATT ACA TGC TCT TTG	799
Ser His Val Ile Leu Leu Gly Ser Thr Val Asn Ile Thr Cys Ser Leu	
30 40 45 50	
30 AAG CCC AGA CAA GGC TGC TTT CAC TAT TCC AGA CGT AAC AAG TTA ATC	847
Lys Pro Arg Gln Gly Cys Phe His Tyr Ser Arg Arg Asn Lys Leu Ile	
35 55 60 65	
35 CTG TAC AAG TTT GAC AGA AGA ATC AAT TTT CAC CAT GGC CAC TCC CTC	895
Leu Tyr Lys Phe Asp Arg Arg Ile Asn Phe His His Gly His Ser Leu	
40 70 75 80 85	
45 AAT TCT CAA GTC ACA GGT CTT CCC CTT GGT ACA ACC TTG TTT GTC TGC	943
Asn Ser Gln Val Thr Gly Leu Pro Leu Gly Thr Thr Leu Phe Val Cys	
45 90 95 100	
50 AAA CTG GCC TGT ATC AAT AGT GAT GAA ATT CAA ATA TGT GGA GCA GAG	991
Lys Leu Ala Cys Ile Asn Ser Asp Glu Ile Gln Ile Cys Gly Ala Glu	
55 105 110 115	

ATC TTC GTT GGT GCT CCA GAA CAG CCT CAA AAT TTA TCC TGC ATA Ile Phe Val Gly Val Ala Pro Glu Gln Pro Gln Asn Leu Ser Cys Ile	5	120	125	130	1039
CAG AAG GGA GAA CAG GGG ACT GTG GCC TGC ACC TGG GAA AGA GGA CGA Gln Lys Gly Glu Gln Gly Thr Val Ala Cys Thr Trp Glu Arg Gly Arg	10	135	140	145	1087
GAC ACC CAC TTA TAC ACT GAG TAT ACT CTA CAG CTA AGT GGA CCA AAA Asp Thr His Leu Tyr Thr Glu Tyr Thr Leu Gln Leu Ser Gly Pro Lys	15	150	155	160	165
AAT TTA ACC TGG CAG AAG CAA TGT AAA GAC ATT TAT TGT GAC TAT TTG Asn Leu Thr Trp Gln Lys Gln Cys Lys Asp Ile Tyr Cys Asp Tyr Leu	20	170	175	180	1183
GAC TTT GGA ATC AAC CTC ACC CCT GAA TCA CCT GAA TCC AAT TTC ACA Asp Phe Gly Ile Asn Leu Thr Pro Glu Ser Pro Glu Ser Asn Phe Thr	25	185	190	195	1231
GCC AAG GTT ACT GCT GTC AAT AGT CTT GGA AGC TCC TCT TCA CTT CCA Ala Lys Val Thr Ala Val Asn Ser Leu Gly Ser Ser Ser Ser Leu Pro	30	200	205	210	1279
TCC ACA TTC ACA TTC TTG GAC ATA GTG AGG CCT CTT CCT CCG TGG GAC Ser Thr Phe Thr Phe Leu Asp Ile Val Arg Pro Leu Pro Pro Trp Asp	35	215	220	225	1327
ATT AGA ATC AAA TTT CAA AAG GCT TCC GTG AGC AGA TGT ACC CTT TAT Ile Arg Ile Lys Phe Gln Lys Ala Ser Val Ser Arg Cys Thr Leu Tyr	40	230	235	240	245
TGG AGA GAT GAG GGA CTG GTA CTG CTT AAT CGA CTC AGA TAT CGG CCC Trp Arg Asp Glu Gly Leu Val Leu Leu Asn Arg Leu Arg Tyr Arg Pro	45				1375
	50				1423

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	250	255	260	
5	AGT AAC AGC AGG CTC TGG AAT ATG GTT AAT GTT ACA AAG GCC AAA GGA Ser Asn Ser Arg Leu Trp Asn Met Val Asn Val Thr Lys Ala Lys Gly			1471
	265	270	275	
10	AGA CAT GAT TTG CTG GAT CTG AAA CCA TTT ACA GAA TAT GAA TTT CAG Arg His Asp Leu Leu Asp Leu Lys Pro Phe Thr Glu Tyr Glu Phe Gln			1519
	280	285	290	
15	ATT TCC TCT AAG CTA CAT CTT TAT AAG GGA AGT TGG AGT GAT TGG AGT Ile Ser Ser Lys Leu His Leu Tyr Lys Gly Ser Trp Ser Asp Trp Ser			1567
20	295	300	305	
25	GAA TCA TTG AGA GCA CAA ACA CCA GAA GAA GAG CCT ACT GGG ATG TTA Glu Ser Leu Arg Ala Gln Thr Pro Glu Glu Glu Pro Thr Gly Met Leu			1615
	310	315	320	325
30	GAT GTC TGG TAC ATG AAA CGG CAC ATT GAC TAC AGT AGA CAA CAG ATT Asp Val Trp Tyr Met Lys Arg His Ile Asp Tyr Ser Arg Gln Gln Ile			1663
	330	335	340	
35	TCT CTT TTC TGG AAG AAT CTG AGT GTC TCA GAG GCA AGA GGA AAA ATT Ser Leu Phe Trp Lys Asn Leu Ser Val Ser Glu Ala Arg Gly Lys Ile			1711
	345	350	355	
40	CTC CAC TAT CAG GTG ACC TTG CAG GAG CTG ACA GGA GGG AAA GCC ATG Leu His Tyr Gln Val Thr Leu Gln Glu Leu Thr Gly Gly Lys Ala Met			1759
	360	365	370	
45	ACA CAG AAC ATC ACA GGA CAC ACC TCC TGG ACC ACA GTC ATT CCT AGA Thr Gln Asn Ile Thr Gly His Thr Ser Trp Thr Thr Val Ile Pro Arg			1807
	375	380	385	

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5	ACC GGA AAT TGG GCT GTG GCT GTG TCT GCA GCA AAT TCA AAA GGC AGT Thr Gly Asn Trp Ala Val Ala Val Ser Ala Ala Asn Ser Lys Gly Ser 390 395 400 405	1855
10	TCT CTG CCC ACT CGT ATT AAC ATA ATG AAC CTG TGT GAG GCA GGG TTG Ser Leu Pro Thr Arg Ile Asn Ile Met Asn Leu Cys Glu Ala Gly Leu 410 415 420	1903
15	CTG GCT CCT CGC CAG GTC TCT GCA AAC TCA GAG GGC ATG GAC AAC ATT Leu Ala Pro Arg Gln Val Ser Ala Asn Ser Glu Gly Met Asp Asn Ile 425 430 435	1951
20	CTG GTG ACT TGG CAG CCT CCC AGG AAA GAT CCC TCT GCT GTT CAG GAG Leu Val Thr Trp Gln Pro Pro Arg Lys Asp Pro Ser Ala Val Gln Glu 440 445 450	1999
25	TAC GTG GTG GAA TGG AGA GAG CTC CAT CCA GGG GGT GAC ACA CAG GTC Tyr Val Val Glu Trp Arg Glu Leu His Pro Gly Gly Asp Thr Gln Val 455 460 465	2047
30	CCT CTA AAC TGG CTA CGG AGT CGA CCC TAC AAT GTG TCT GCT CTG ATT Pro Leu Asn Trp Leu Arg Ser Arg Pro Tyr Asn Val Ser Ala Leu Ile 470 475 480 485	2095
35	TCA GAG AAC ATA AAA TCC TAC ATC TGT TAT GAA ATC CGT GTG TAT GCA Ser Glu Asn Ile Lys Ser Tyr Ile Cys Tyr Glu Ile Arg Val Tyr Ala 490 495 500	2143
45	CTC TCA GGG GAT CAA GGA GGA TGC AGC TCC ATC CTG GGT AAC TCT AAG Leu Ser Gly Asp Gln Gly Cys Ser Ser Ile Leu Gly Asn Ser Lys 505 510 515	2191
50	CAC AAA GCA CCA CTG AGT GGC CCC CAC ATT AAT CCC ATC ACA GAG GAA His Lys Ala Pro Leu Ser Gly Pro His Ile Asn Ala Ile Thr Glu Glu	2239

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	520	525	530	
5	AAG GGG AGC ATT TTA ATT TCA TGG AAC AGC ATT CCA GTC CAG GAG CAA			2287
	Lys Gly Ser Ile Leu Ile Ser Trp Asn Ser Ile Pro Val Gln Glu Gln			
	535	540	545	
10	ATG GGC TGC CTC CTC CAT TAT AGG ATA TAC TGG AAG GAA CGG GAC TCC			2335
	Met Gly Cys Leu Leu His Tyr Arg Ile Tyr Trp Lys Glu Arg Asp Ser			
15	550	555	560	565
	AAC TCC CAG CCT CAG CTC TGT GAA ATT CCC TAC AGA GTC TCC CAA AAT			2383
	Asn Ser Gln Pro Gln Leu Cys Glu Ile Pro Tyr Arg Val Ser Gln Asn			
20	570	575	580	
	TCA CAT CCA ATA AAC AGC CTG CAG CCC CGA GTG ACA TAT GTC CTG TGG			2431
25	Ser His Pro Ile Asn Ser Leu Gln Pro Arg Val Thr Tyr Val Leu Trp			
	585	590	595	
30	ATG ACA GCT CTG ACA GCT GGT GAA AGT TCC CAC GGA AAT GAG AGG			2479
	Met Thr Ala Leu Thr Ala Ala Gly Glu Ser Ser His Gly Asn Glu Arg			
	600	605	610	
35	GAA TTT TGT CTG CAA GGT AAA GCC AAT TGG ATG GCG TTT GTG GCA CCA			2527
	Glu Phe Cys Leu Gln Gly Lys Ala Asn Trp Met Ala Phe Val Ala Pro			
	615	620	625	
40	AGC ATT TGC ATT GCT ATC ATC ATG GTG GCC ATT TTC TCA ACG CAT TAC			2575
	Ser Ile Cys Ile Ala Ile Ile Met Val Gly Ile Phe Ser Thr His Tyr			
	630	635	640	645
45	TTC CAG CAA AAG GTG TTT GTT CTC CTA GCA GCC CTC AGA CCT CAG TGG			2623
	Phe Gln Gln Lys Val Phe Val Leu Leu Ala Ala Leu Arg Pro Gln Trp			
50	650	655	660	

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EP 0 759 466 A2

TGT AGC AGA GAA ATT CCA GAT CCA GCA AAT AGC ACT TGC GCT AAG AAA Cys Ser Arg Glu Ile Pro Asp Pro Ala Asn Ser Thr Cys Ala Lys Lys	5	665	670	675	2671
TAT CCC ATT GCA GAG GAG AAG ACA CAG CTG CCC TTG GAC AGG CTC CTG Tyr Pro Ile Ala Glu Glu Lys Thr Gln Leu Pro Leu Asp Arg Leu Leu	10	680	685	690	2719
ATA GAC TGG CCC ACG CCT GAA GAT CCT GAA CCG CTG GTC ATC AGT GAA Ile Asp Trp Pro Thr Pro Glu Asp Pro Glu Pro Leu Val Ile Ser Glu	15	695	700	705	2767
GTC CTT CAT CAA GTG ACC CCA GTT TTC AGA CAT CCC CCC TGC TCC AAC Val Leu His Gln Val Thr Pro Val Phe Arg His Pro Pro Cys Ser Asn	20	710	715	720	2815
TGG CCA CAA AGG GAA AAA GGA ATC CAA GGT CAT CAG GCC TCT GAG AAA Trp Pro Gln Arg Glu Lys Gly Ile Gln Gly His Gln Ala Ser Glu Lys	25	730	735	740	2863
GAC ATG ATG CAC AGT GCC TCA AGC CCA CCT CCA AGA GCT CTC CAA Asp Met Met His Ser Ala Ser Ser Pro Pro Pro Arg Ala Leu Gln	30	745	750	755	2911
GCT GAG AGC AGA CAA CTG GTG GAT CTG TAC AAG GTG CTG GAG AGC AGG Ala Glu Ser Arg Gln Leu Val Asp Leu Tyr Lys Val Leu Glu Ser Arg	35	760	765	770	2959
GGC TCC GAC CCA AAG CCA GAA AAC CCA GCC TGT CCC TGG ACG GTG CTC Gly Ser Asp Pro Lys Pro Glu Asn Pro Ala Cys Pro Trp Thr Val Leu	40	775	780	785	3007
CCA GCA GGT GAC CTT CCC ACC CAT GAT GGC TAC TTA CCC TCC AAC ATA Pro Ala Gly Asp Leu Pro Thr His Asp Gly Tyr Leu Pro Ser Asn Ile	45				3055

56

790	795	800	805	
5 GAT GAC CTC CCC TCA CAT GAG GCA CCT CTC GCT GAC TCT CTG GAA GAA Asp Asp Leu Pro Ser His Glu Ala Pro Leu Ala Asp Ser Leu Glu Glu				3103
810 815 820				
10 CTG GAG CCT CAG CAC ATC TCC CTT TCT GTT TTC CCC TCA AGT TCT CTT Leu Glu Pro Gln His Ile Ser Leu Ser Val Phe Pro Ser Ser Ser Leu				3151
825 830 835				
15 CAC CCA CTC ACC TTC TCC TGT GGT GAT AAG CTG ACT CTG GAT CAG TTA His Pro Leu Thr Phe Ser Cys Gly Asp Lys Leu Thr Leu Asp Gln Leu				3199
840 845 850				
20 AAC ATG AGG TGT GAC TCC CTC ATG CTC TGAGTGGTGA GGCTTCAAGC Lys Met Arg Cys Asp Ser Leu Met Leu				3246
855 860				
25 CTTAAAGTCA GTGTGCCCTC AACCAGCACA GCCTGCCCA ATTCCCCAG CCCCTGCTCC				3306
30 AGCAGCTGTC ATCTCTGGGT GCCACCATCG GTCTGGCTGC AGCTAGAGGA CAGGCAAGCC				3366
35 AGCTCTGGGG GAGTCTTAGG AACTGGGAGT TGGTCTTCAC TCAGATGCCT CATCTTGCT				3426
40 TTCCCAGGGC CTTAAAATTA CATCCTTCAC TGTGTGGACC TAGAGACTCC AACTTGAATT				3486
45 CCTAGTAACT TTCTTGGTAT GCTGGCCAGA AAGGGAAATG AGGAGGAGAG TAGAAAACCAC				3546
AGCTCTTAGT AGTAATGGCA TACAGTCTAG AGGACCATTG ATGCAATGAC TATTTCTAAA				3606
50 GCACCTGCTA CACAGCAGGC TGTACACAGC AGATCAGTAC TGTTAACAG AACTTCCCTGA				3666
GATGATGGAA ATGTTCTACC TCTGCACTCA CTGTCCAGTA CATTAGACAC TAGGCACATT				3726

GGCTGTTAAT CACTTGAAT GTGTTAGCT TGACTGAGGA ATTAATTTT GATTGTAAT	3786
5 TTAAATCGCC ACACATGGCT AGTGGCTACT GTATTGGAGT GCACAGCTCT AGATGGCTCC	3846
TAGATTATTG AGAGCCTCCA AAACAAATCA ACCTAGTTCT ATAGATGAAG ACATAAAAGA	3906
10 CACTGGTAAA CACCAATGTA AAAGGGCCCC CAAGGTGGTC ATGACTGGTC TCATTTGCAG	3966
AAGTCTAAGA ATGTACCTTT TTCTGGCCGG GCGTGGTAGC TCATGCCTGT AATCCCAGCA	4026
15 CTTTGGGAGG CTGA	4040

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(2) INFORMATION FOR SEQ ID NO:2:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 862 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: protein

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala His Thr Phe Arg Gly Cys Ser Leu Ala Phe Met Phe Ile Ile	
40 1 5 10 15	

Thr Trp Leu Leu Ile Lys Ala Lys Ile Asp Ala Cys Lys Arg Gly Asp	
45 20 25 30	

Val Thr Val Lys Pro Ser His Val Ile Leu Leu Gly Ser Thr Val Asn	
35 40 45	

50 Ile Thr Cys Ser Leu Lys Pro Arg Gln Gly Cys Phe His Tyr Ser Arg

55

	50	55	60
5	Arg Asn Lys Leu Ile Leu Tyr Lys Phe Asp Arg Arg Ile Asn Phe His		
	65	70	75
10	His Gly His Ser Leu Asn Ser Gln Val Thr Gly Leu Pro Leu Gly Thr		
	85	90	95
15	Thr Leu Phe Val Cys Lys Leu Ala Cys Ile Asn Ser Asp Glu Ile Gln		
	100	105	110
20	Ile Cys Gly Ala Glu Ile Phe Val Gly Val Ala Pro Glu Gln Pro Gln		
	115	120	125
25	Asn Leu Ser Cys Ile Gln Lys Gly Glu Gln Gly Thr Val Ala Cys Thr		
	130	135	140
30	Trp Glu Arg Gly Arg Asp Thr His Leu Tyr Thr Glu Tyr Thr Leu Gln		
	145	150	155
35	Leu Ser Gly Pro Lys Asn Leu Thr Trp Gln Lys Gln Cys Lys Asp Ile		
	165	170	175
40	Tyr Cys Asp Tyr Leu Asp Phe Gly Ile Asn Leu Thr Pro Glu Ser Pro		
	180	185	190
45	Glu Ser Asn Phe Thr Ala Lys Val Thr Ala Val Asn Ser Leu Gly Ser		
	195	200	205
50	Ser Ser Ser Leu Pro Ser Thr Phe Thr Phe Leu Asp Ile Val Arg Pro		
	210	215	220
55	Leu Pro Pro Trp Asp Ile Arg Ile Lys Phe Gln Lys Ala Ser Val Ser		
	225	230	235
			240

Arg Cys Thr Leu Tyr Trp Arg Asp Glu Gly Leu Val Leu Leu Asn Arg
 245 250 255

5
 Leu Arg Tyr Arg Pro Ser Asn Ser Arg Leu Trp Asn Met Val Asn Val
 260 265 270

10
 Thr Lys Ala Lys Gly Arg His Asp Leu Leu Asp Leu Lys Pro Phe Thr
 275 280 285

15
 Glu Tyr Glu Phe Gln Ile Ser Ser Lys Leu His Leu Tyr Lys Gly Ser
 290 295 300

20
 Trp Ser Asp Trp Ser Glu Ser Leu Arg Ala Gln Thr Pro Glu Glu Glu
 305 310 315 320

25
 Pro Thr Gly Met Leu Asp Val Trp Tyr Met Lys Arg His Ile Asp Tyr
 325 330 335

30
 Ser Arg Gln Gln Ile Ser Leu Phe Trp Lys Asn Leu Ser Val Ser Glu
 340 345 350

35
 Ala Arg Gly Lys Ile Leu His Tyr Gln Val Thr Leu Gln Glu Leu Thr
 355 360 365

40
 Gly Gly Lys Ala Met Thr Gln Asn Ile Thr Gly His Thr Ser Trp Thr
 370 375 380

45
 Thr Val Ile Pro Arg Thr Gly Asn Trp Ala Val Ala Val Ser Ala Ala
 385 390 395 400

50
 Asn Ser Lys Gly Ser Ser Leu Pro Thr Arg Ile Asn Ile Met Asn Leu
 405 410 415

Cys Glu Ala Gly Leu Leu Ala Pro Arg Gln Val Ser Ala Asn Ser Glu
 420 425 430

5
 Gly Met Asp Asn Ile Leu Val Thr Trp Gln Pro Pro Arg Lys Asp Pro
 435 440 445

10
 Ser Ala Val Gln Glu Tyr Val Val Glu Trp Arg Glu Leu His Pro Gly
 450 455 460

15
 Gly Asp Thr Gln Val Pro Leu Asn Trp Leu Arg Ser Arg Pro Tyr Asn
 465 470 475 480

20
 Val Ser Ala Leu Ile Ser Glu Asn Ile Lys Ser Tyr Ile Cys Tyr Glu
 485 490 495

25
 Ile Arg Val Tyr Ala Leu Ser Gly Asp Gln Gly Gly Cys Ser Ser Ile
 500 505 510

30
 Leu Gly Asn Ser Lys His Lys Ala Pro Leu Ser Gly Pro His Ile Asn
 515 520 525

35
 Ala Ile Thr Glu Glu Lys Gly Ser Ile Leu Ile Ser Trp Asn Ser Ile
 530 535 540

40
 Pro Val Gln Glu Gln Met Gly Cys Leu Leu His Tyr Arg Ile Tyr Trp
 545 550 555 560

45
 Lys Glu Arg Asp Ser Asn Ser Gln Pro Gln Leu Cys Glu Ile Pro Tyr
 565 570 575

50
 Arg Val Ser Gln Asn Ser His Pro Ile Asn Ser Leu Gln Pro Arg Val
 580 585 590

55
 Thr Tyr Val Leu Trp Met Thr Ala Leu Thr Ala Ala Gly Glu Ser Ser

	595	600	605
5	His Gly Asn Glu Arg Glu Phe Cys Leu Gln Gly Lys Ala Asn Trp Met		
	610	615	620
10	Ala Phe Val Ala Pro Ser Ile Cys Ile Ala Ile Ile Met Val Gly Ile		
	625	630	635
	Phe Ser Thr His Tyr Phe Gln Gln Lys Val Phe Val Leu Leu Ala Ala		
15	645	650	655
20	Leu Arg Pro Gln Trp Cys Ser Arg Glu Ile Pro Asp Pro Ala Asn Ser		
	660	665	670
25	Thr Cys Ala Lys Lys Tyr Pro Ile Ala Glu Glu Lys Thr Gln Leu Pro		
	675	680	685
30	Leu Asp Arg Leu Leu Ile Asp Trp Pro Thr Pro Glu Asp Pro Glu Pro		
	690	695	700
35	Leu Val Ile Ser Glu Val Leu His Gln Val Thr Pro Val Phe Arg His		
	705	710	715
	Pro Pro Cys Ser Asn Trp Pro Gln Arg Glu Lys Gly Ile Gln Gly His		
40	725	730	735
45	Gln Ala Ser Glu Lys Asp Met Met His Ser Ala Ser Ser Pro Pro Pro		
	740	745	750
50	Pro Arg Ala Leu Gln Ala Glu Ser Arg Gln Leu Val Asp Leu Tyr Lys		
	755	760	765
	Val Leu Glu Ser Arg Gly Ser Asp Pro Lys Pro Glu Asn Pro Ala Cys		
	770	775	780

Pro Trp Thr Val Leu Pro Ala Gly Asp Leu Pro Thr His Asp Gly Tyr
 785 790 795 800

5

Leu Pro Ser Asn Ile Asp Asp Leu Pro Ser His Glu Ala Pro Leu Ala
 805 810 815

10

Asp Ser Leu Glu Glu Leu Glu Pro Gln His Ile Ser Leu Ser Val Phe
 820 825 830

15

Pro Ser Ser Ser Leu His Pro Leu Thr Phe Ser Cys Gly Asp Lys Leu
 835 840 845

20

Thr Leu Asp Gln Leu Lys Met Arg Cys Asp Ser Leu Met Leu
 850 855 860

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(2) INFORMATION FOR SEQ ID NO:3:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2104 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: cDNA to mRNA

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(iii) HYPOTHETICAL: NO

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(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (G) CELL TYPE: human T-cells

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(vii) IMMEDIATE SOURCE:

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- (A) LIBRARY: library 3 day PHA/pEF-BOS
 (B) CLONE: human interleukin-12 receptor clone #5

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(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 65..2050

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GGTGGCTGAA CCTCGCAGGT GGCAGAGAGG CTCCCTGGG GCTGTGGGC TCTACGTGGA	60
20 TCCG ATG GAG CCG CTG GTG ACC TGG GTG GTC CCC CTC CTC TTC CTC TTC	109
Met Glu Pro Leu Val Thr Trp Val Val Pro Leu Leu Phe Leu Phe	
1 5 10 15	
25 CTG CTG TCC AGG CAG GGC GCT GCC TGC AGA ACC AGT GAG TGC TGT TTT	157
Leu Leu Ser Arg Gln Gly Ala Ala Cys Arg Thr Ser Glu Cys Cys Phe	
20 25 30	
30 CAG GAC CCG CCA TAT CCG GAT GCA GAC TCA GGC TCG GCC TCG GGC CCT	205
Gln Asp Pro Pro Tyr Pro Asp Ala Asp Ser Gly Ser Ala Ser Gly Pro	
35 40 45	
40 AGG GAC CTG AGA TGC TAT CCG ATA TCC AGT GAT CGT TAC GAG TGC TCC	253
Arg Asp Leu Arg Cys Tyr Arg Ile Ser Ser Asp Arg Tyr Glu Cys Ser	
50 55 60	
45 TGG CAG TAT GAG GGT CCC ACA GCT GGG GTC AGC CAC TTC CTG CGG TGT	301
Trp Gln Tyr Glu Gly Pro Thr Ala Gly Val Ser His Phe Leu Arg Cys	
65 70 75	
50 TGC CTT AGC TCC GGG CGC TGC TAC TTC GCC GCC GGC TCA GCC ACC	349
Cys Leu Ser Ser Gly Arg Cys Cys Tyr Phe Ala Ala Gly Ser Ala Thr	

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	80	85	90	95	
5	AGG CTG CAG TTC TCC GAC CAG GCT GGG GTG TCT GTG CTG TAC ACT GTC				397
	Arg Leu Gln Phe Ser Asp Gln Ala Gly Val Ser Val Leu Tyr Thr Val				
	100	105	110		
10					
	ACA CTC TGG GTG GAA TCC TGG GCC AGG AAC CAG ACA GAG AAG TCT CCT				445
	Thr Leu Trp Val Glu Ser Trp Ala Arg Asn Gln Thr Glu Lys Ser Pro				
15	115	120	125		
20	GAG GTG ACC CTC CAG CTC TAC AAC TCA GTT AAA TAT GAG CCT CCT CTG				493
	Glu Val Thr Leu Gln Leu Tyr Asn Ser Val Lys Tyr Glu Pro Pro Leu				
	130	135	140		
25					
	GGA GAC ATC AAG GTG TCC AAG TTG GCC GGG CAG CTG CGT ATG GAG TGG				541
	Gly Asp Ile Lys Val Ser Lys Leu Ala Gly Gln Leu Arg Met Glu Trp				
	145	150	155		
30					
	GAG ACC CCG GAT AAC CAG GTT GGT GCT GAG GTG CAG TTC CGG CAC CGG				589
	Glu Thr Pro Asp Asn Gln Val Gly Ala Glu Val Gln Phe Arg His Arg				
	160	165	170	175	
35					
	ACA CCC AGC AGC CCA TGG AAG TTG GGC GAC TGC GGA CCT CAG GAT GAT				637
	Thr Pro Ser Ser Pro Trp Lys Leu Gly Asp Cys Gly Pro Gln Asp Asp				
	180	185	190		
40					
	GAT ACT GAG TCC TGC CTC TGC CCC CTG GAG ATG AAT GTG GCC CAG GAA				685
	Asp Thr Glu Ser Cys Leu Cys Pro Leu Glu Met Asn Val Ala Gln Glu				
45	195	200	205		
50	TTC CAG CTC CGA CGA CGG CAG CTG GGG AGC CAA GGA AGT TCC TGG AGC				733
	Phe Gln Leu Arg Arg Gln Leu Gly Ser Gln Gly Ser Ser Trp Ser				
	210	215	220		

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AAG TGG AGC AGC CCC GTG TGC GTT CCC CCT GAA AAC CCC CCA CAG CCT Lys Trp Ser Ser Pro Val Cys Val Pro Pro Glu Asn Pro Pro Gln Pro	781
5 225 230 235.	
 CAG GTG AGA TTC TCG GTG GAG CAG CTG GGC CAG GAT GGG AGG AGG CGG 10 Gln Val Arg Phe Ser Val Glu Gln Leu Gly Gln Asp Gly Arg Arg Arg	829
240 245 250 255	
 CTG ACC CTG AAA GAG CAG CCA ACC CAG CTG GAG CTT CCA GAA GGC TGT 15 Leu Thr Leu Lys Glu Gln Pro Thr Gln Leu Glu Leu Pro Glu Gly Cys	877
260 265 270	
 CAA GGG CTG GCG CCT GGC ACG GAG GTC ACT TAC CGA CTA CAG CTC CAC 20 Gln Gly Leu Ala Pro Gly Thr Glu Val Thr Tyr Arg Leu Gln Leu His	925
275 280 285	
 25 ATG CTG TCC TGC CCG TGT AAG GCC AAG GCC ACC AGG ACC CTG CAC CTG Met Leu Ser Cys Pro Cys Lys Ala Lys Ala Thr Arg Thr Leu His Léu	973
290 295 300	
 30 GGG AAG ATG CCC TAT CTC TCG GGT GCT GCC TAC AAC GTG GCT GTC ATC Gly Lys Met Pro Tyr Leu Ser Gly Ala Ala Tyr Asn Val Ala Val Ile	1021
35 305 310 315	
 40 TCC TCG AAC CAA TTT GGT CCT GGC CTG AAC CAG ACG TGG CAC ATT CCT Ser Ser Asn Gln Phe Gly Pro Gly Leu Asn Gln Thr Trp His Ile Pro	1069
320 325 330 335	
 45 GCC GAC ACC CAC ACA GAA CCA GTG GCT CTG AAT ATC AGC GTC GGA ACC Ala Asp Thr His Thr Glu Pro Val Ala Leu Asn Ile Ser Val Gly Thr	1117
340 345 350	
 50 AAC GGG ACC ACC ATG TAT TGG CCA GCC CGG GCT CAG AGC ATG ACG TAT Asn Gly Thr Thr Met Tyr Trp Pro Ala Arg Ala Gln Ser Met Thr Tyr	1165

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5	TGC ATT GAA TGG CAG CCT GTG GGC CAG GAC GGG GGC CTT GCC ACC TGC Cys Ile Glu Trp Gln Pro Val Gly Gln Asp Gly Gly Leu Ala Thr Cys		1213	
	370	375	380	
10	AGC CTG ACT GCG CCG CAA GAC CCG GAT CCG GCT GGA ATG GCA ACC TAC Ser Leu Thr Ala Pro Gln Asp Pro Asp Pro Ala Gly Met Ala Thr Tyr		1261	
	385	390	395	
15	AGC TGG AGT CGA GAG TCT GGG GCA ATG GGG CAG GAA AAG TGT TAC TAC Ser Trp Ser Arg Glu Ser Gly Ala Met Gly Gln Glu Lys Cys Tyr Tyr		1309	
	400	405	410	415
20	ATT ACC ATC TTT GCC TCT GCG CAC CCC GAG AAG CTC ACC TTG TGG TCT Ile Thr Ile Phe Ala Ser Ala His Pro Glu Lys Leu Thr Leu Trp Ser		1357	
	420	425	430	
25	ACG GTC CTG TCC ACC TAC CAC TTT GGG GGC AAT GCC TCA GCA GCT GGG Thr Val Leu Ser Thr Tyr His Phe Gly Gly Asn Ala Ser Ala Ala Gly		1405	
	435	440	445	
30	ACA CCG CAC CAC GTC TCG GTG AAG AAT CAT AGC TTG GAC TCT GTG TCT Thr Pro His His Val Ser Val Lys Asn His Ser Leu Asp Ser Val Ser		1453	
	450	455	460	
35	GTG GAC TGG GCA CCA TCC CTG CTG AGC ACC TGT CCC GGC GTC CTA AAG Val Asp Trp Ala Pro Ser Leu Leu Ser Thr Cys Pro Gly Val Leu Lys		1501	
	465	470	475	
40	GAG TAT GTT GTC CGC TGC CGA GAT GAA GAC AGC AAA CAG GTG TCA GAG Glu Tyr Val Val Arg Cys Arg Asp Ser Lys Gln Val Ser Glu		1549	
	480	485	490	495
45				
50				
55				

	CAT CCC GTG CAG CCC ACA GAG ACC CAA GTT ACC CTC AGT GGC CTG CGG		1597
	His Pro Val Gln Pro Thr Glu Thr Gln Val Thr Leu Ser Gly Leu Arg		
5	500	505	510
	GCT GGT GTA GCC TAC ACG GTG CAG GTG CGA GCA GAC ACA GCG TGG CTG		1645
10	Ala Gly Val Ala Tyr Thr Val Gln Val Arg Ala Asp Thr Ala Trp Leu		
	515	520	525
15	AGG GGT GTC TGG AGC CAG CCC CAG CGC TTC AGC ATC GAA GTG CAG GTT		1693
	Arg Gly Val Trp Ser Gln Pro Gln Arg Phe Ser Ile Glu Val Gln Val		
	530	535	540
20	TCT GAT TGG CTC ATC TTC TTC GCC TCC CTG GGG AGC TTC CTG AGC ATC		1741
	Ser Asp Trp Leu Ile Phe Phe Ala Ser Leu Gly Ser Phe Leu Ser Ile		
	545	550	555
25	CTT CTC GTG GGC GTC CTT GGC TAC CTT GGC CTG AAC AGG GCC GCA CGG		1789
	Leu Leu Val Gly Val Leu Gly Tyr Leu Gly Leu Asn Arg Ala Ala Arg		
	560	565	570
30	575		
	CAC CTG TGC CCG CCG CTG CCC ACA CCC TGT GCC AGC TCC GCC ATT GAG		1837
	His Leu Cys Pro Pro Leu Pro Thr Pro Cys Ala Ser Ser Ala Ile Glu		
35	580	585	590
	TTC CCT GGA GGG AAG GAG ACT TGG CAG TGG ATC AAC CCA GTG GAC TTC		1885
40	Phe Pro Gly Gly Lys Glu Thr Trp Gln Trp Ile Asn Pro Val Asp Phe		
	595	600	605
45	CAG GAA GAG GCA TCC CTG CAG GAG GCC CTG GTG GTA GAG ATG TCC TGG		1933
	Gln Glu Glu Ala Ser Leu Gln Glu Ala Leu Val Val Glu Met Ser Trp		
	610	615	620
50	GAC AAA GGC GAG AGG ACT GAG CCT CTC GAG AAG ACA GAG CTA CCT GAG		1981
	Asp Lys Gly Glu Arg Thr Glu Pro Leu Glu Lys Thr Glu Leu Pro Glu		

625	630	635	
5 GGT GCC CCT GAG CTG GCC CTG GAT ACA GAG TTG TCC TTG GAG GAT GGA Gly Ala Pro Glu Leu Ala Leu Asp Thr Glu Leu Ser Leu Glu Asp Gly			2029
640	645	650	655
10 GAC AGG TGC AAG GCC AAG ATG TGATCGTTGA GGCTCAGAGA GGGTGAGTGA Asp Arg Cys Lys Ala Lys Met			2080
15 660			
20 CTCGCCCGAG GCTACGTAGC CTTT			2104

(2) INFORMATION FOR SEQ ID NO:4:

- 25 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 662 amino acids
 - (B) TYPE: amino acid
 - 30 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- 35 (ix) FEATURE:
- (A) NAME/KEY: Region
 - 40 (B) LOCATION: 1..20
 - (D) OTHER INFORMATION: /note= "N-terminal signal peptide
(1..20 or 23 or 24)"

- 45 (ix) FEATURE:
- (A) NAME/KEY: Region
 - 50 (B) LOCATION: 541..570
 - (D) OTHER INFORMATION: /note= "transmembrane region"

5 (ix) FEATURE:

- (A) NAME/KEY: Region
(B) LOCATION: 571..662
(D) OTHER INFORMATION: /note= "cytoplasmic tail region"

10 (ix) FEATURE:

- (A) NAME/KEY: Region
(B) LOCATION: 52..64
(D) OTHER INFORMATION: /note= "sequence motif of cytokine
15 receptor superfamily Cys52..Cys62SW"

20 (ix) FEATURE:

- (A) NAME/KEY: Region
(B) LOCATION: 222..226
(D) OTHER INFORMATION: /note= "cytokine receptor
25 superfamily motif (W222SKWS)"

30 (ix) FEATURE:

- (A) NAME/KEY: Region
(B) LOCATION: 121..123
(D) OTHER INFORMATION: /note= "N-linked glycosylation
35 site"

40 (ix) FEATURE:

- (A) NAME/KEY: Region
(B) LOCATION: 329..331
(D) OTHER INFORMATION: /note= "N-linked glycosylation
45 site"

50 (ix) FEATURE:

- (A) NAME/KEY: Region
(B) LOCATION: 346..348
(D) OTHER INFORMATION: /note= "N-linked glycosylation

site"

5 (ix) FEATURE:

- (A) NAME/KEY: Region
(B) LOCATION: 352..354
10 (D) OTHER INFORMATION: /note= "N-linked glycosylation
site"

15 (ix) FEATURE:

- (A) NAME/KEY: Region
(B) LOCATION: 442..444
20 (D) OTHER INFORMATION: /note= "N-linked glycosylation
site"

25 (ix) FEATURE:

- (A) NAME/KEY: Region
(B) LOCATION: 456..458
30 (D) OTHER INFORMATION: /note= "N-linked glycosylation
site"

35 (ix) FEATURE:

- (A) NAME/KEY: Region
(B) LOCATION: 24..540
(D) OTHER INFORMATION: /note= "Extracellular region"

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

45 Met Glu Pro Leu Val Thr Trp Val Val Pro Leu Leu Phe Leu
1 5 10 15

50 Leu Ser Arg Gln Gly Ala Ala Cys Arg Thr Ser Glu Cys Cys Phe Gln
20 25 30

55

Asp Pro Pro Tyr Pro Asp Ala Asp Ser Gly Ser Ala Ser Gly Pro Arg

35 40 45

5

Asp Leu Arg Cys Tyr Arg Ile Ser Ser Asp Arg Tyr Glu Cys Ser Trp

50 55 60

10

Gln Tyr Glu Gly Pro Thr Ala Gly Val Ser His Phe Leu Arg Cys Cys

65 70 75 80

15

Leu Ser Ser Gly Arg Cys Cys Tyr Phe Ala Ala Gly Ser Ala Thr Arg

85 90 95

20

Leu Gln Phe Ser Asp Gln Ala Gly Val Ser Val Leu Tyr Thr Val Thr

100 105 110

25

Leu Trp Val Glu Ser Trp Ala Arg Asn Gln Thr Glu Lys Ser Pro Glu

115 120 125

30

Val Thr Leu Gln Leu Tyr Asn Ser Val Lys Tyr Glu Pro Pro Leu Gly

130 135 140

35

Asp Ile Lys Val Ser Lys Leu Ala Gly Gln Leu Arg Met Glu Trp Glu

145 150 155 160

40

Thr Pro Asp Asn Gln Val Gly Ala Glu Val Gln Phe Arg His Arg Thr

165 170 175

45

Pro Ser Ser Pro Trp Lys Leu Gly Asp Cys Gly Pro Gln Asp Asp Asp

180 185 190

50

Thr Glu Ser Cys Leu Cys Pro Leu Glu Met Asn Val Ala Gln Glu Phe

195 200 205

55

Gln Leu Arg Arg Gln Leu Gly Ser Gln Gly Ser Ser Trp Ser Lys

210	215	220	
5 Trp Ser Ser Pro Val Cys Val Pro Pro Glu Asn Pro Pro Gln Pro Gln			
225	230	235	240
10 Val Arg Phe Ser Val Glu Gln Leu Gly Gln Asp Gly Arg Arg Arg Leu			
	245	250	255
15 Thr Leu Lys Glu Gln Pro Thr Gln Leu Glu Leu Pro Glu Gly Cys Gln			
	260	265	270
20 Gly Leu Ala Pro Gly Thr Glu Val Thr Tyr Arg Leu Gln Leu His Met			
	275	280	285
25 Leu Ser Cys Pro Cys Lys Ala Lys Ala Thr Arg Thr Leu His Leu Gly			
	290	295	300
30 Lys Met Pro Tyr Leu Ser Gly Ala Ala Tyr Asn Val Ala Val Ile Ser			
	305	310	315
35 Ser Asn Gln Phe Gly Pro Gly Leu Asn Gln Thr Trp His Ile Pro Ala			
	325	330	335
40 Asp Thr His Thr Glu Pro Val Ala Leu Asn Ile Ser Val Gly Thr Asn			
	340	345	350
45 Gly Thr Thr Met Tyr Trp Pro Ala Arg Ala Gln Ser Met Thr Tyr Cys			
	355	360	365
50 Ile Glu Trp Gln Pro Val Gly Gln Asp Gly Gly Leu Ala Thr Cys Ser			
	370	375	380
55 Leu Thr Ala Pro Gln Asp Pro Asp Pro Ala Gly Met Ala Thr Tyr Ser			
	385	390	395
			400

Trp Ser Arg Glu Ser Gly Ala Met Gly Gln Glu Lys Cys Tyr Tyr Ile
 405 410 415

5
 Thr Ile Phe Ala Ser Ala His Pro Glu Lys Leu Thr Leu Trp Ser Thr
 420 425 430

10
 Val Leu Ser Thr Tyr His Phe Gly Gly Asn Ala Ser Ala Ala Gly Thr
 435 440 445

15
 Pro His His Val Ser Val Lys Asn His Ser Leu Asp Ser Val Ser Val
 450 455 460

20
 Asp Trp Ala Pro Ser Leu Leu Ser Thr Cys Pro Gly Val Leu Lys Glu
 465 470 475 480

25
 Tyr Val Val Arg Cys Arg Asp Ser Lys Gln Val Ser Glu His
 485 490 495

30
 Pro Val Gln Pro Thr Glu Thr Gln Val Thr Leu Ser Gly Leu Arg Ala
 500 505 510

35
 Gly Val Ala Tyr Thr Val Gln Val Arg Ala Asp Thr Ala Trp Leu Arg
 515 520 525

40
 Gly Val Trp Ser Gln Pro Gln Arg Phe Ser Ile Glu Val Gln Val Ser
 530 535 540

45
 Asp Trp Leu Ile Phe Phe Ala Ser Leu Gly Ser Phe Leu Ser Ile Leu
 545 550 555 560

50
 Leu Val Gly Val Leu Gly Tyr Leu Gly Leu Asn Arg Ala Ala Arg His
 565 570 575

Leu Cys Pro Pro Leu Pro Thr Pro Cys Ala Ser Ser Ala Ile Glu Phe

580 585

590

5

Pro Gly Gly Lys Glu Thr Trp Gln Trp Ile Asn Pro Val Asp Phe Gln

595 600

605

10

Glu Glu Ala Ser Leu Gln Glu Ala Leu Val Val Glu Met Ser Trp Asp

610 615

620

15

Lys Gly Glu Arg Thr Glu Pro Leu Glu Lys Thr Glu Leu Pro Glu Gly

625 630 635

640

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Ala Pro Glu Leu Ala Leu Asp Thr Glu Leu Ser Leu Glu Asp Gly Asp

645 650

655

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Arg Cys Lys Ala Lys Met

660

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Claims

1. A low binding affinity interleukin-12 (IL-12) beta2 receptor protein, or a fragment thereof which

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- (a) has low binding affinity for IL-12, and
- (b) when complexed with a IL-12 beta1 receptor protein forms a complex having high binding affinity to IL-12.

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2. The protein of claim 1, wherein the IL-12 beta2 receptor protein is encoded by a nucleic acid having a sequence that hybridises under stringent conditions to nucleic acid sequence SEQ ID NO:1.

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3. The protein of claim 2 which shares at least 80% sequence homology with the polypeptide having the SEQ ID NO:2.

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4. The protein of claim 3, wherein the IL-12 beta2 receptor protein has SEQ ID NO:2 or allelic forms or variants thereof.

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5. The protein of any one of claims 1 to 4 encoded by a nucleic acid which comprises two subsequences, wherein one of said subsequences encodes a soluble protein as defined in any one of the preceding claims, and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of Ig other than the first domain of said constant region.

6. A complex capable of binding to IL-12 with high affinity, comprising interleukin-12 (IL-12) beta2 receptor protein, or a fragment thereof as defined in any of claims 1 - 4 complexed with IL-12 beta1 receptor protein, or a fragment thereof which

- (a) has low binding affinity for IL-12, and
- (b) when complexed with a IL-12 beta2 receptor protein forms a complex having high binding affinity to IL-12.

7. The complex of claim 6, wherein the IL-12 beta1 receptor protein is encoded by a nucleic acid having a sequence that hybridises under stringent conditions to nucleic acid sequence SEQ ID NO:3.
8. The protein of claim 7 which shares at least 80% sequence homology with the polypeptide having the SEQ ID NO:4.
9. The protein of claim 8, wherein the IL-12 beta1 receptor protein has SEQ ID NO:4 or allelic forms or variants thereof.
10. A protein encoded by a first and a second nucleic acid, wherein the first nucleic acid comprises two subsequences, wherein one of said subsequences encodes a soluble fragment of any one of claims 1 to 4 and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region, and the second nucleic acid comprises two subsequences wherein one of said subsequences encodes a soluble fragment of a protein of any of claims 7 to 9 and the other of said subsequences encodes all of the domains of the constant region of the heavy chain of human Ig other than the first domain of said constant region.
11. A protein or complex of any one of claims 1 to 10 which is soluble.
12. Nucleic acids which encode a protein or complex of any one of claims 1 - 11.
13. The nucleic acid of claim 12, wherein the nucleic acid which encodes human IL-12 beta2 receptor protein having the SEQ ID NO:1.
14. The nucleic acid of claim 12, wherein the nucleic acid which encodes human IL-12 beta1 receptor protein having the SEQ ID NO:3.
15. A vector comprising a nucleic acid of any one of claims 12 to 14.
16. An expression vector comprising a nucleic acid of any one of claims 12 - 14 operably linked to control sequences recognised by a host cell.
17. A host cell transformed with a nucleic acid of any one of claims 12 to 16.
18. The host cell of claim 17 wherein the protein or complex is expressed on its surface.
19. The host cell of claim 18 wherein the host cell proliferates in the presence of IL-12.
20. The host cell of claims 17 - 19, wherein the host cell is transformed with a first vector comprising a nucleic acid encoding the protein as defined in claim 1 and a second vector comprising a nucleic acid encoding the protein as defined in claim 7 or with a single vector comprising a nucleic acid encoding the protein as defined in claim 1 and a nucleic acid encoding a protein as defined in claim 7.
21. An antibody directed against a protein of any of claims 1 to 11.
22. A process for the preparation of a protein of any of claims 1 to 11 which comprises the expression of a nucleic acid of any one of claims 12 to 14 in a suitable host cell.
23. A pharmaceutical composition comprising a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22 or the antibody of claim 21 and a pharmaceutically acceptable carrier.
24. The pharmaceutical composition of claim 23 which further comprises a therapeutically effective amount of one or more cytokine antagonists.
25. The use of a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22 or the antibody of claim 21 for the preparation of a medicament.
26. The use of a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22 or the antibody of claim 21 for the preparation of a medicament for the treatment of autoimmune dysfunction.

27. A method for screening compounds useful for inhibition of IL-12 activity, comprising

- a) contacting a compound suspected of inhibiting IL-12 activity to a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22, and
5 b) detection of the inhibiting effect.

28. A method for screening compounds useful as agonists of IL-12, comprising

- a) contacting a compound suspected of being an IL-12 agonist to a protein or complex of any one of claims 1 to 11 or as obtained by the process of claim 22, and
10 b) detection of an agonist effect.

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Fig. 1

10 20 30 40 50 60
 TGCAGAGAAC AGAGAAAGGA CATCTGCGAG GAAAGTTCCC TGATGGCTGT CAACAAAGTG
 70 80 90 100 110 120
 CCACGTCTCT ATGGCTGTGT ACGCTGAGCA CACGATTTA TCGCGCCTAT CATATCTTGG
 130 140 150 160 170 180
 TGCATAAAACG CACCTCACCT CGGTCAACCC TTGCTCCGTC TTATGAGACA GGCTTTATTA
 190 200 210 220 230 240
 TCCGCATTT ATATGAGGGG AATCTGACGG TGGAGAGAGA ATTATCTTGC TCAAGGGCAC
 250 260 270 280 290 300
 ACAGCAGAGC CCACAGGTGG CAGAATCCCA CCGGAGCCCCG CTTCGACCCG. CGGGGTGGAA
 310 320 330 340 350 360
 ACCACGGGCG CCCGCCCGGC TGCGCTTCCA GAGCTGAACG GAGAAGCGAG TCCTCTCCGC
 370 380 390 400 410 420
 CCTGCGGCCA CCGCCCGAGCC CCGACCCCCCG CCCCAGCCCCG ATCCTCACTC GCCGCCAGCT
 430 440 450 460 470 480
 CCCCGCGCCC ACCCCGGAGT TGGTGGCGCA GAGGCGGGAG GCGGAGGCAG GAGGGCGGGC
 490 500 510 520 530 540
 GCTGGCACCG GGAACGCCCG AGCGCCGGCA GAGAGCGCGG AGAGCGCGAC ACGTGCGGCC
 550 560 570 580 590 600
 CAGAGCACCG GGGCCACCCCG GTCCCCGCAG GCCCAGGACC GCGCCCGCTG GCAGGGCGACA
 610 620 630 640 650 660
 CGTGGAAAGAA TACGGAGTTC TATACCAGAG TTGATTGTTG ATGGCACATA CTTTTAGAGG
 670 680 690 700 710 720
 ATGCTCATTC GCATTTATGT TTATAATCAC GTGGCTGTTG ATTTAAAGCAA AAATAGATGC
 730 740 750 760 770 780
 GTGCAAGAGA GGCAGATGTGA CTGTGAAGCC TTCCCATGTA ATTTTACTTG GATCCACTGT
 790 800 810 820 830 840
 CAATATTACA TGCTCTTGA AGCCAGACA AGGCTGCTT CACTATTCCA GACGTAACAA
 850 860 870 880 890 900
 GTTAATCCCG TACAAGTTTG ACAGAAGAAT CAATTTCAC CATGGCCACT CCCTCAATTG
 910 920 930 940 950 960
 TCAAGTCACA GGTCTTCCCC TTGGTACAAC CTTGTTGTC TGCAAACCTGG CCTGTATCAA
 970 980 990 1000 1010 1020
 TAGTGATGAA ATTCAAAATAT GTGGAGCAGA GATCTTCGTT GGTGTTGCTC CAGAACAGCC

Fig. 1 CONT'D

1030 1040 1050 1060 1070 1080
 TCAAAATTAA TCCTGCATAC AGAAGGGAGA ACAGGGACT GTGGCCTGCA CCTGGGAAAG

 1090 1100 1110 1120 1130 1140
 AGGACGAGAC ACCCACTTAT AACTGAGTA TACTCTACAG CTAAGTGGAC CAAAAAAATTAA

 1150 1160 1170 1180 1190 1200
 AACCTGGCAG AAGCAATGTA AAGACATTAA TTGTGACTAT TTGGACTTTG GAATCAACCT

 1210 1220 1230 1240 1250 1260
 CACCCCTGAA TCACCTGAAT CCAATTTCAC AGCCAAGGTT ACTGCTGTCA ATAGTCTTGG

 1270 1280 1290 1300 1310 1320
 AAGCTCCTCT TCACCTCCAT CCACATTACAC ATTCTTGGAC ATAGTGAGGC CTCTTCCTCC

 1330 1340 1350 1360 1370 1380
 GTGGGACATT AGAACATAAT TTCAAAAGGC TTCCGTGAGC AGATGTACCC TTTATTGGAG

 1390 1400 1410 1420 1430 1440
 AGATGAGGGG CTGGTACTGC TTAATCGACT CAGATATCGG CCCAGTAACA GCAGGCTCTG

 1450 1460 1470 1480 1490 1500
 GAATATGGTT AATGTTACAA AGGCCAAAGG AAGACATGAT TTGCTGGATC TGAAACCATT

 1510 1520 1530 1540 1550 1560
 TACAGAAATAT GAATTCAGA TTTCTCTAA GCTACATCTT TATAAGGGAA GTTGGAGTGA

 1570 1580 1590 1600 1610 1620
 TTGGAGTGAA TCATTGAGAG CACAAACACC AGAAGAAGAG CCTACTGGGA TGTTAGATGT

 1630 1640 1650 1660 1670 1680
 CTGGTACATG AAACGGCACA TTGACTACAG TAGACAAACAG ATTTCTCTTT TCTGGAAGAA

 1690 1700 1710 1720 1730 1740
 TCTGAGTGTC TCAGAGGCAA GAGGAAAAT TCTCCACTAT CAGGTGACCT TGCAGGAGCT

 1750 1760 1770 1780 1790 1800
 GACAGGAGGG AAAGCCATGA CACAGAACAT CACAGGACAC ACCTCCTGGA CCACAGTCAT

 1810 1820 1830 1840 1850 1860
 TCCTAGAACCG GGAAATTGGG CTGTGGCTGT GTCTGCAGCA AATTCAAAAG GCAGTTCTCT

 1870 1880 1890 1900 1910 1920
 GCCCACTCGT ATTAACATAA TGAACCTGTG TGAGGCAGGG TTGCTGGCTC CTCGCCAGGT

 1930 1940 1950 1960 1970 1980
 CTCTGCAAAAC TCAGAGGGCA TGGACAAACAT TCTGGTACT TGGCAGCCTC CCAGGAAACA

 1990 2000 2010 2020 2030 2040
 TCCCTCTGCT GTTCAGGAGT ACGTGGTGGGA ATGGAGAGAG CTCCATCCAG GGGGTGACAC

Fig. 1 CONT'D

2050 2060 2070 2080 2090 2100
 ACAGGTCCCT CTAAACTGGC TACGGAGTCG ACCCTACAAT GTGTCTGCTC TGATTTCAAGA

 2110 2120 2130 2140 2150 2160
 GAACATAAAA TCCTACATCT GTTATGAAAT CCGTGTGTAT GCACTCTCAG GGGATCAAGG

 2170 2180 2190 2200 2210 2220
 AGGATGCAGC TCCATCCTGG GTAACCTCAA GCACAAAGCA CCACGTAGTG GCCCCCACAT

 2230 2240 2250 2260 2270 2280
 TAATGCCATC ACAGAGGAAA AGGGGAGCAT TTTAATTCA TGGAACAGCA TTCCAGTCCA

 2290 2300 2310 2320 2330 2340
 GGAGCAAATG GGCTGCCCTCC TCCATTATAG GATATACTGG AAGGAACGGG ACTCCAACTC

 2350 2360 2370 2380 2390 2400
 CCAGGCCTCAG CTCTGTGAAA TTCCCTACAG AGTCTCCCAA AATTACACATC CAATAAACAG

 2410 2420 2430 2440 2450 2460
 CCTGCAGCCC CGAGTGACAT ATGTCCTGTG GATGACAGCT CTGACAGCTG CTGGTAAAG

 2470 2480 2490 2500 2510 2520
 TTCCCACGGA AATGAGAGGG AATTTTGCTCT GCAAGGTAAA GCCAATTGGA TGGCGTTGT

 2530 2540 2550 2560 2570 2580
 GGCACCAAGC ATTTGCATIG CTATCATCAT GGTGGGCATT TTCTCAACGC ATTACTTCCA

 2590 2600 2610 2620 2630 2640
 GCAAAAGGTG TTTGTTCTCC TAGCAGCCCT CAGACCTCAG TGGTAGCA GAGAAATTCC

 2650 2660 2670 2680 2690 2700
 AGATCCAGCA AATAGCACTT GCGCTAAGAA ATATCCATT GCAGAGGAGA AGACACAGCT

 2710 2720 2730 2740 2750 2760
 GCCCTTGGAC AGGCTCTGA TAGACTGGCC CACGCCCTGAA GATCCTGAAC CGCTGGTCAT

 2770 2780 2790 2800 2810 2820
 CAGTGAAGTC CTTCATCAAG TGACCCCCAGT TTTCAGACAT CCCCCCTGCT CCAACTGGCC

 2830 2840 2850 2860 2870 2880
 ACAAAAGGGAA AAAGGAATCC AAGGTCTCA GGCCTCTGAG AAAGACATGA TGCACAGTGC

 2890 2900 2910 2920 2930 2940
 CTCAGGCCCA CCACCTCCAA GAGCTCTCCA AGCTGAGAGC AGACAACTGG TGGATCTGTA

 2950 2960 2970 2980 2990 3000
 CAAGGTGCTG GAGAGCAGGG GCTCCGACCC AAAGCCAGAA AACCCAGCCT GTCCCTGGAC

 3010 3020 3030 3040 3050 3060
 GGTGCTCCCA GCAGGTGACC TTCCCAACCA TGATGGCTAC TTACCCCTCCA ACATAGATGA

Fig. 1 CONT'D

3070 3080 3090 3100 3110 3120
 CCTCCCCCTCA CATGAGGCAC CTCTCGCTGA CTCTCTGGAA GAACTGGAGC CTCAGCACAT

 3130 3140 3150 3160 3170 3180
 CTCCCTTCT GTTTTCCCCT CAAGTTCTCT TCACCCACTC ACCTTCTCCT GTGGTGATAA

 3190 3200 3210 3220 3230 3240
 GCTGACTCTG GATCAGTTAA AGATGAGGTG TGACTCCCTC ATGCTCTGAG TGGTGAGGCT

 3250 3260 3270 3280 3290 3300
 TCAAGCCTTA AAGTCAGTGT GCCCTCAACC AGCACAGCCT GCCCCAATTG CCCCAGCCCC

 3310 3320 3330 3340 3350 3360
 TGCTCCAGCA GCTGTCATCT CTGGGTGCCA CCATCGGTCT GGCTGCAGCT AGAGGACAGG

 3370 3380 3390 3400 3410 3420
 CAAGCCAGCT CTGGGGGAGT CTTAGGAACG GGGAGTTGGT CTTCACTCAG ATGCCTCATC

 3430 3440 3450 3460 3470 3480
 TTGCCTTCC CAGGGCCTTA AAATTACATC CTTCACTGTG TGGACCTAGA GACTCCAATC

 3490 3500 3510 3520 3530 3540
 TGAATTCTTA GTAACTTTCT TGGTATGCTG GCCAGAAAGG GAAATGAGGA GGAGAGTAGA

 3550 3560 3570 3580 3590 3600
 AACCCACAGCT CTTAGTAGTA ATGGCATAACA GTCTAGAGGA CCATTCTATGC AATGACTATT

 3610 3620 3630 3640 3650 3660
 TCTAAAGCAC CTGCTACACA GCAGGCTGTA CACAGCAGAT CAGTACTGTT CAACAGAACT

 3670 3680 3690 3700 3710 3720
 TCCTGAGATG ATGGAAATGT TCTACCTCTG CACTCACTGT CCAGTACATT AGACACTAGG

 3730 3740 3750 3760 3770 3780
 CACATTGGCT GTTAATCACT TGGAAATGTGT TTAGCTTGAC TGAGGAATTA AATTTTGATT

 3790 3800 3810 3820 3830 3840
 GTAAATTTAA ATCGCCACAC ATGGCTAGTG GCTACTGTAT TGGAGTGCAC AGCTCTAGAT

 3850 3860 3870 3880 3890 3900
 GGCTCCTAGA TTATTGAGAG CCTCCAAAAC AAATCAACCT AGTTCTATAG ATGAAGACAT

 3910 3920 3930 3940 3950 3960
 AAAAGACACT GGTAAACACC AATGTAAAAG GGCCCCCAAG GTGGTCATGA CTGGTCTCAT

 3970 3980 3990 4000 4010 4020
 TTGCAGAAGT CTAAGAATGT ACCTTTTCT GGCGGGCGT GGTAGCTCAT GCCTGTAATC

 4030 4040
 CCAGCACTTT GGGAGGCTGA

Fig. 2

1 MAHTFRGCSL AFMFIITWLL IKAKIDACKR GDVTVKPSHV ILLGSTVNIT
 51 CSLKPRQGCF HYSRRNKLIL YKFDRRINFH HGHSLSNSQVT GLPLGTTLFV
 101 CKLACINSDE IQICGAEIFV GVAPEQPQNL SCIQKGEQGT VACTWERGRD
 151 THLYTEYTLQ LSGPKNLTWQ KQCKDIYCDY LDFGINLTPE SPESNFTAKV
 201 TAVNSLGSSS SLPSTFTFLD IVRPLPPWDI RIKFQKASVS RCTLYWRDEG
 251 LVLLNRLRYR PSNSRLWNMV NVTKAKGRHD LLDLKPFTTEY EFQISSKLHL
 301 YKGWSDWSE SLRAQTPEEE PTGMLDVWYM KRHIDYSRQQ ISLFWKNLSV
 351 SEARGKILHY QVTLQELTGG KAMTQVITGH TSWTTVIPRT GNWAVAVSAA
 401 NSKGSSSLPTR INIMNLCEAG LLAPRQVSAN SEGMDNILVT WQPPRKDPSA
 451 VQEYVVEWRE LHPGGDTQVP LNWLRSRPYN VSALISENIK SYICYEIRVY
 501 ALSGDQGGCS SILGNSKHKA PLSGPHINAI TEEKGSILIS WNSIPVQEQM
 551 GCLLHYRIYW KERDSNSQPQ LCEIPYRVSQ NSHPINSLQP RVTYVLWMTA
 601 LTAAGESSHG NEREFCLQGK ANWMAFVAPS ICIAIIMVGI FSTHYFQQKV
 651 FVLLAALRPQ WCSREIPDPA NSTCAKKYPI AEEKTQLPLD RLLIDWPTPE
 701 DPERPLVISEV LHQVTPVFRH PPCSNWPQRE KGIQGHQASE KDMMHSASSP
 751 PPPRALQAES RQLVDLYKVL ESRGSDPKPE NPACPWTVLP AGDLPTHDGY
 801 LPSNIDDLPS HEAPLADSLE ELEPQHISLS VFPSSSLHPL TFSCGDKLTL
 851 DQLKMRCDSL ML

Fig. 3

10 20 30 40 50 60 70
 CCTGGCTGAA CCTCGCAGGT GGCAAGAGGG CTCCCTGGG GCTGTGGGGC TCTACGTGGA TCCGATGGAG
 80 90 100 110 120 130 140
 CCCGCTGGTGA CCTGGGTGGT CCCCCCTCTC TTCCCTCTCC TGCTGTCCAG GCAGGGCGCT GCCTGCAGAA
 150 160 170 180 190 200 210
 CCAGTGAGTG CTGTTTCAG GACCCGCCAT ATCCGGATGC AGACTCAGGC TCGGCCTCGG GCCCTAGGGA
 220 230 240 250 260 270 280
 CCTGAGATGC TATCGGATAT CCAGTGATCG TTACCGAGTCC TCTGGCAGT ATGAGGGTCC CACAGCTGGG
 290 300 310 320 330 340 350
 CTCAGCCACT TCCCTGGGTG TTGCCTTAGC TCCGGGGCCT GCTGCTACTT CGCCGCCGGC TCAGCCACCA
 360 370 380 390 400 410 420
 GCGTGCAGTT CTCCGACCAAG GCTGGGGTGT CTGTGCTGTA CACTGTCACA CTCTGGGTGG AATCCTGGG
 430 440 450 460 470 480 490
 CAGGAACCAAG ACAGAGAAGT CTCCTGAGGT GACCCCTGCAG CTCTACAACT CAGTTAAATA TGAGCCTCCT
 500 510 520 530 540 550 560
 CTGGGAGACA TCAAGGTGTC CAAGTTGGCC GGGCAGCTGC GTATGGAGTG GGAGACCCCG GATAACCAGG
 570 580 590 600 610 620 630
 TTGGTGCCTGA CGTGCAGTTG CGGCACCGGA CACCCAGCAG CCCATGGAAG TTGGGGCAGT GCGGACCTCA
 640 650 660 670 680 690 700
 GGATGATGAT ACTGAGTCCT GCCTCTGCC CCGTGGAGATG AATGTGGCCC AGGAATTCCA GCTCCGACCA
 710 720 730 740 750 760 770
 CGGCAGCTGG GGAGCCAAAGG AAGTCTCTGG AGCAAGTGG ACGAGCCCGT GTGGCTTCCC CCTGAAAACC
 780 790 800 810 820 830 840
 CCCCACAGCC TCAGGTGAGA TTCTGGTGG AGCAGCTGGG CCAGGATGGG AGGAGGGGGC TGACCCCTGAA
 850 860 870 880 890 900 910
 AGAGCAGCCA ACCCAGCTGG AGCTTCCAGA AGGCTGTCAA GGGCTGGGC CTGGCACCGA GGTCACTIAC
 920 930 940 950 960 970 980
 CGACTACAGC TCCACATGCT CTCCCTGCCG TGTAAAGGCCA AGGCCACCCAG GACCCCTGCAC CTGGGGAAAGA
 990 1000 1010 1020 1030 1040 1050
 TGCCTATCT CTGGGGTGTG CTGCTACACG TGGCTGTCACT CTCCTCGAAC CAATTTGGTC CTGGCTGAA
 1060 1070 1080 1090 1100 1110 1120
 CCAGACGTGG CACATTCTG CGCACACCCA CACAGAACCA GTGGCTCTGA ATATCAGCGT CGGAACCAAC

Fig. 3 CONT'D

1130 1140 1150 1160 1170 1180 1190
 GGGACCACCA TGTATTGGCC AGCCCCGGCT CAGAGCATGA CGTATTGCAT TGAATGGCAG CCTGTGGGCC
 1200 1210 1220 1230 1240 1250 1260
 AGGACGGGGG CCTTGCCACC TGCAGCCTGA CTGCGCCGCA AGACCCGGAT CCGGCTGGAA TGGCAACCTA
 1270 1280 1290 1300 1310 1320 1330
 CAGCTGGAGT CGAGAGTC TG GGGCAATGGG GCAGGAAAG TGTTACTACA TTACCATCTT TGCCCTCTGG
 1340 1350 1360 1370 1380 1390 1400
 CACCCCGAGA AGCTCACCTT GTGGCTACG GTCCCTGTCCA CCTACCACTT TGGGGGCAAT GCCTCAGCAG
 1410 1420 1430 1440 1450 1460 1470
 CTGGGACACC GCACCAACGTC TCGGTGAAGA ATCATAGCTT GGACTCTGTG TCTGTGGACT GGGCACCATC
 1480 1490 1500 1510 1520 1530 1540
 CCTGCTGAGC ACCTGTCCCG GCGTCCTAAA GGAGTATGTT GTCCGCTGCC GAGATGAAGA CAGCAAACAG
 1550 1560 1570 1580 1590 1600 1610
 GTGTCAGAGC ATCCCCGTGCA GCCCACAGAG ACCCAAGTTA CCCTCACTGG CCTGGGGCT GGCTGTAGCCT
 1620 1630 1640 1650 1660 1670 1680
 ACACGGTGCA GGTGCGAGCA GACACAGCGT GGCTGAGGGG TGTCCTGGAGC CAGCCCCAGC GCTTCAGCAT
 1690 1700 1710 1720 1730 1740 1750
 CGAAAGTGCAG GTTTCTGATT GGCTCATCTT CTTCGGCTCC CTGGGGAGCT TCCTGAGCAT CCTTCTCGTG
 1760 1770 1780 1790 1800 1810 1820
 GCGGTCTTIG GCTACCTTGG CCTGAACAGG GCGGCACGGC ACCTGTGCC GCGGTGCC ACACCCGTG
 1830 1840 1850 1860 1870 1880 1890
 CCAGCTCCGC CATTGAGTC CCTGGAGGGG AGGAGACTTG CGACTGGATC AACCCAGTGG ACTTCCAGGA
 1900 1910 1920 1930 1940 1950 1960
 AGAGGCATCC CTGCAGGGGG CCCTGGTGGT AGAGATGTCC TGGGACAAAG GCGAGAGGAC TGAGCTCTC
 1970 1980 1990 2000 2010 2020 2030
 GAGAAGACAG AGCTACCTGA GGGTCCCCCT GAGCTGGCCC TGGATACAGA GTTGTCTTG GAGGATGGAG
 2040 2050 2060 2070 2080 2090 2100
 ACAGGTGCAA GGCAAGATG TGATCGTTGA GGCTCAGAGA GGGTAGTGA CTGGCCCCAG GCTACGTAGC

Fig. 4

10 20 30 40 50 60 70
 MERLYTWVVP LFFELLSRQ GAACRTSECC FQDPYPDAD SGASGPRDL RCYRISSDRY ECSWQYEGPT
 80 90 100 110 120 130 140
 AGVSHFLRCC LSSGRCCYFA AGSATRLQFS DQAGVSVLYT VTLWVESWAR NQTEKSPEVT LQLYNSVKYE
 150 160 170 180 190 200 210
 PPLGDIKVSK LAGQLRMWEV TPDNQVGAEV QFRHRTPSSP WKLGDCGPQD DDTESCLCPL EMNVQAQEFLQ
 220 230 240 250 260 270 280
 RRRQLGSQGS SWSKWSSPVC VPPENPPQQ VRFSEVQLQQ DGRRLTLKE QPTQLELPEG CQGLAPGTEV
 290 300 310 320 330 340 350
 TYRLQLHMLS CPCKAKATRT LHLGKMPYLS GAAYNNAVIS SNQFGPGLNQ TWHIPADTHT EPVALNISVG
 360 370 380 390 400 410 420
 TNGTTMYWPA RAQSMTYCIE WQPVGQDGGL ATCSLTAPQD PDPMAGMATYS WSRESGAMGQ EKCYXYITIFA
 430 440 450 460 470 480 490
 SAHPEKTLW STVLSTYHFG. GNASAAGTPH HVSVKNHISLD SVSVDWAPSL LSTCPGVLK EYVVRCRDED
 500 510 520 530 540 550 560
 KQVSEHPVQP TETQVTLSQL RAGVAYTVQV RADTAWLRGV WSQQPQRFSIE VQVSDWLIFF ASLGFSFLSIL
 570 580 590 600 610 620 630
 LVGVLYGL GL MRAARHILCPP LPUPCASSAI EFPGGKETWQ WINPVDFQEE ASLQEALYVE MSWDKGERTE
 640 650 660
 PLEKTELPEG APELALDTTEL SLEDGDRCKA KH

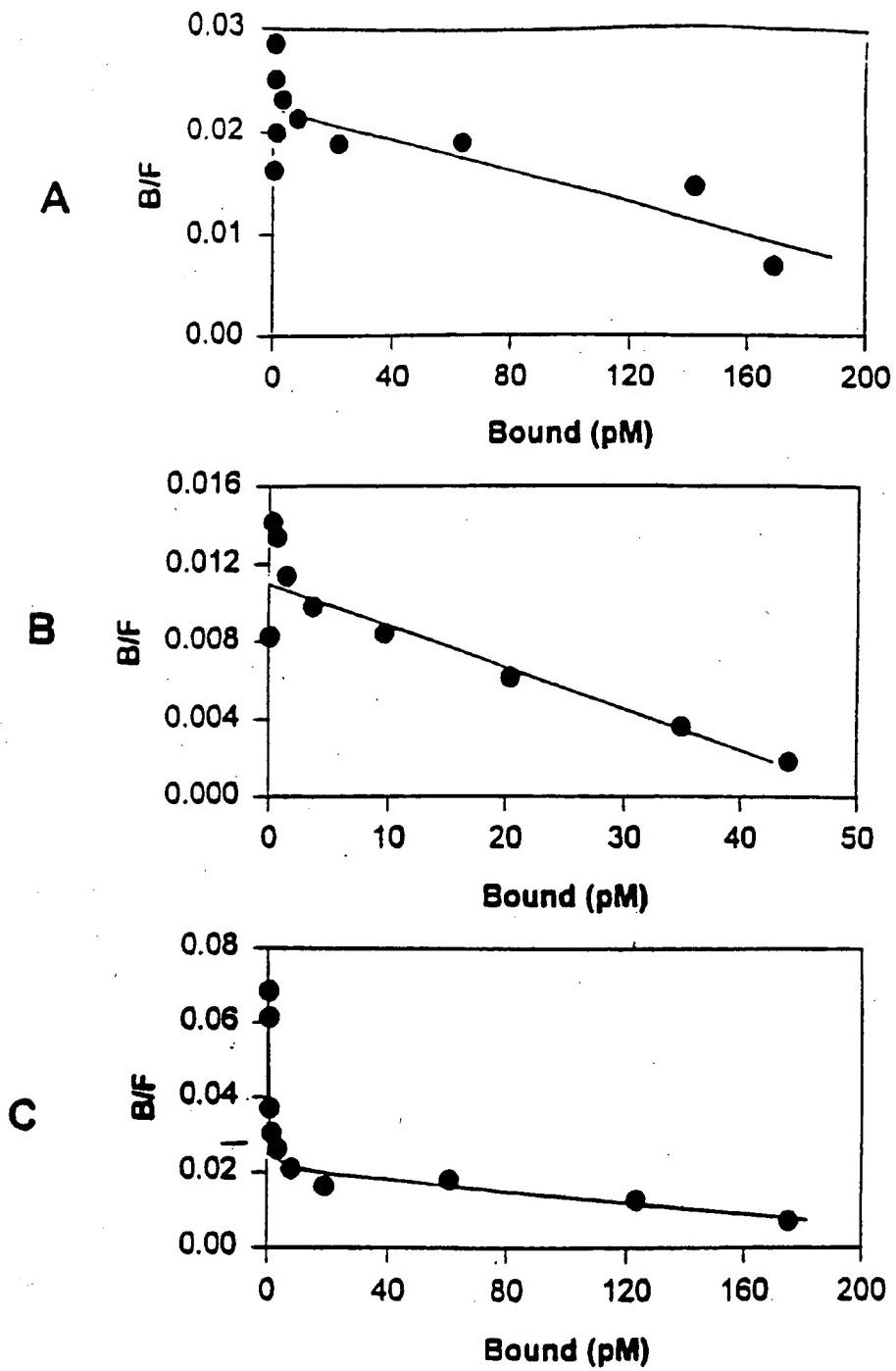
Fig. 5

Fig. 6

